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The invention provides a composition comprising a polypeptide and hydrochlorothiazide and triamterene covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Hydrochlorothiazide and triamterene preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting hydrochlorothiazide and triamterene from degradation comprising covalently attaching it to a polypeptide.

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The invention also provides a method for delivering hydrochlorothiazide and triamterene to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, hydrochlorothiazide and triamterene are released from the composition by an enzyme-catalyzed release. In another preferred embodiment, hydrochlorothiazide and triamterene are released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and hydrochlorothiazide and triamterene are released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, hydrochlorothiazide and triamterene are released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, hydrochlorothiazide and triamterene are released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching hydrochlorothiazide and triamterene to a side chain of an amino acid to form an active agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, hydrochlorothiazide and triamterene and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis

of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent
5 is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

10 It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

15 DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize hydrochlorothiazide and triamterene and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of hydrochlorothiazide and triamterene. Furthermore, active agents can be
20 combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

The composition of the invention comprises hydrochlorothiazide and triamterene covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide,
25 (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the
5 conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding
10 are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best
15 model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with
20 protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and
25 contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

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Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

5 As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the
10 kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

15 Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention
20 has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

25 As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to

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poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

5 The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal
10 enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using
15 any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart
20 specific properties to the drug delivery system.

 The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these
25 polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the
30 alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are
5 known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, hydrochlorothiazide and triamterene are covalently attached to the polypeptide via the amine group on each.

The polypeptide carrier can be prepared using conventional techniques. A
10 preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG)
15 and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal
20 epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the
25 transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the

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mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-hydrochlorothiazide and triamterene conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

15 Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

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Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

15 Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

 γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 hydrochlorothiazide and triamterene covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein hydrochlorothiazide and triamterene are
covalently attached to a side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

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12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein hydrochlorothiazide and triamterene are conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing hydrochlorothiazide and triamterene from said composition in a pH-dependent manner.

15 19. A method for protecting hydrochlorothiazide and triamterene from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of hydrochlorothiazide and triamterene from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching hydrochlorothiazide and triamterene to said polypeptide.

20 21. A method for delivering hydrochlorothiazide and triamterene to a patient comprising administering to said patient a composition comprising:

 a polypeptide; and

 hydrochlorothiazide and triamterene covalently attached to said polypeptide.

25 22. The method of claim 21 wherein hydrochlorothiazide and triamterene are released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein hydrochlorothiazide and triamterene are released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

10 **Abstract**

 A composition comprising a polypeptide and hydrochlorothiazide and triamterene covalently attached to the polypeptide. Also provided is a method for delivery of hydrochlorothiazide and triamterene to a patient comprising administering to the patient a composition comprising a polypeptide and hydrochlorothiazide and triamterene
15 covalently attached to the polypeptide. Also provided is a method for protecting hydrochlorothiazide and triamterene from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of hydrochlorothiazide and triamterene from a composition comprising covalently attaching it to the polypeptide.

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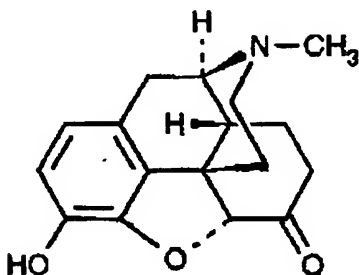
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING HYDROMORPHONE AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to hydromorphone, as well as methods for protecting and administering hydromorphone. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a
10 known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

 Hydromorphone is a known pharmaceutical agent that is used in the treatment of
15 cough and pain. Its structure is:



 The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered
20 product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

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Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase
5 markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly
10 acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the
15 active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and
20 aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can
25 also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage

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reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in

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the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients.

Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (hydromorphone) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching hydromorphone to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising hydromorphone microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and hydromorphone covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Hydromorphone preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a

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carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting hydromorphone from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering hydromorphone to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, hydromorphone is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, hydromorphone is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and hydromorphone is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, hydromorphone is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, hydromorphone is

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released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

(a) attaching hydromorphone to a side chain of an amino acid to form an active agent/amino acid complex;

(b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and

(c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, hydromorphone and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is

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described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

5 The present invention provides several benefits for active agent delivery. First, the invention can stabilize hydromorphone and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of hydromorphone. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

10 The composition of the invention comprises hydromorphone covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or
15 more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the
20 conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding
25 are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded

protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational
5 entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular
10 bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has
15 maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is
20 a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious
25 chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

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Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

5 Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

10 Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

15 Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

20 As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

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Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant

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groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence,

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maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action
5 for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is
10 distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or
15 the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

20 In the present invention, hydromorphone is covalently attached to the polypeptide via the hydroxyl group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer
25 can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the

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invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal
5 epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the
10 transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active
15 agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the
20 catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-hydromorphone conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

25 Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

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Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5 several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10 followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15 the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20 a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
25 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 hydromorphone covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein hydromorphone is covalently attached to
a side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

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12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein hydromorphone is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing hydromorphone from said composition in a pH-dependent manner.

15 19. A method for protecting hydromorphone from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of hydromorphone from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching hydromorphone to said polypeptide.

20 21. A method for delivering hydromorphone to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
hydromorphone covalently attached to said polypeptide.

25 22. The method of claim 21 wherein hydromorphone is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein hydromorphone is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

- 5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

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Abstract

A composition comprising a polypeptide and hydromorphone covalently attached to the polypeptide. Also provided is a method for delivery of hydromorphone to a patient comprising administering to the patient a composition comprising a polypeptide and
5 hydromorphone covalently attached to the polypeptide. Also provided is a method for protecting hydromorphone from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of hydromorphone from a composition comprising covalently attaching it to the polypeptide.

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**A NOVEL PHARMACEUTICAL COMPOUND CONTAINING
HYDROXYCHLOROQUINONE AND METHODS OF
MAKING AND USING SAME**

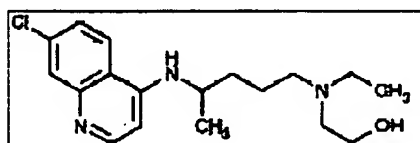
5 FIELD OF THE INVENTION

The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to hydroxychloroquinone, as well as methods for protecting and administering hydroxychloroquinone. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of

10 taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

15 Hydroxychloroquinone is a known pharmaceutical agent that is used in the treatment of malaria. Its structure is:



The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability

20 of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

25 Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken

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under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even
5 reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of
10 cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release
20 through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several
25 shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent

in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

- 5 In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral
- 10 administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group.
- 15 This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the
- 20 active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

- It is also important to control the molecular weight, molecular size and particle
- 25 size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients.
- 30 Particle size not only becomes a problem with injectable drugs, as in the HAR

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application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent
5 (hydroxychloroquinone) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching hydroxychloroquinone to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the
10 stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

15 Alternatively, the present invention provides a pharmaceutical composition comprising hydroxychloroquinone microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and hydroxychloroquinone covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally
20 occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Hydroxychloroquinone preferably is covalently attached to a side chain, the N-
25 terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent

is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a
5 microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet,
10 an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting hydroxychloroquinone from
15 degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering hydroxychloroquinone to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, hydroxychloroquinone is
20 released from the composition by an enzyme-catalyzed release. In another preferred embodiment, hydroxychloroquinone is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and hydroxychloroquinone is released from the composition by dissolution of the microencapsulating agent. In another
25 preferred embodiment, hydroxychloroquinone is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, hydroxychloroquinone is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled

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by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method

5 comprises the steps of:

(a) attaching hydroxychloroquinone to a side chain of an amino acid to form an active agent/amino acid complex;

(b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and

10 (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, hydroxychloroquinone and a second active agent can be copolymerized in step (c).

15 In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side
20 chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

25 It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize hydroxychloroquinone and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of hydroxychloroquinone. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

The composition of the invention comprises hydroxychloroquinone covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

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The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be

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enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will
5 ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of
10 the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a
15 synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level
20 of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond
25 between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate

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weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border
 5 membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

10

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For
 15 example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could
 20 conceivably have a loading of 58%, although this may not be entirely practical.

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The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

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The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, hydroxychloroquinone is covalently attached to the polypeptide via the hydroxyl group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized
5 adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions,
10 sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is
15 particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

20 Preferably, the resultant peptide-hydroxychloroquinone conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

25 An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product

precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

- The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C.
- 5 The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

- 10 In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product
- 15 is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

- Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include
- 20 dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

- There have been over 30 different γ -alkyl glutamates prepared any one of which
- 25 may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for

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several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C.

- 5 The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

- 10 γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly(γ -Alkyl Glutamate)

- 15 γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

- 20 Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

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CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 hydroxychloroquinone covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein hydroxychloroquinone is covalently attached to a side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar and a salt.
11. The composition of claim 1 further comprising an adjuvant.

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12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein hydroxychloroquinone is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing hydroxychloroquinone from said composition in a pH-dependent manner.

15 19. A method for protecting hydroxychloroquinone from degradation comprising covalently attaching said active agent to a polypeptide.

20 20. A method for controlling release of hydroxychloroquinone from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching hydroxychloroquinone to said polypeptide.

20 21. A method for delivering hydroxychloroquinone to a patient comprising administering to said patient a composition comprising:
 a polypeptide; and
 hydroxychloroquinone covalently attached to said polypeptide.

25 22. The method of claim 21 wherein hydroxychloroquinone is released from said composition by an enzyme-catalyzed release.

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23. The method of claim 21 wherein hydroxychloroquinone is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

- 5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and hydroxychloroquinone covalently attached to the polypeptide. Also provided is a method for delivery of hydroxychloroquinone to a patient comprising administering to the patient a composition comprising a polypeptide and hydroxychloroquinone covalently attached to the polypeptide. Also provided is a method for protecting hydroxychloroquinone from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of hydroxychloroquinone from a composition comprising covalently attaching it to the polypeptide.

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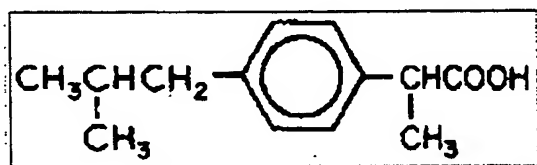
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING IBUPROFEN AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to ibuprofen, as well as methods for protecting and administering ibuprofen. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

 Ibuprofen is a known pharmaceutical agent that is used in the treatment of pain
15 and arthritis. Its structure is:



 The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered
20 product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

 Active agent delivery systems are often critical for the effective delivery of a
25 biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken

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under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even
5 reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of
10 cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

15 Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release
20 through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several
25 shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent

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in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

5 In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral
10 administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group.
15 This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the
20 active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

 It is also important to control the molecular weight, molecular size and particle
25 size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients.
30 Particle size not only becomes a problem with injectable drugs, as in the HAR

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application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent
5 (ibuprofen) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching ibuprofen to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through
10 conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

15 Alternatively, the present invention provides a pharmaceutical composition comprising ibuprofen microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and ibuprofen covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a
20 heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Ibuprofen preferably is covalently attached to a side chain, the N-terminus or the
25 C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is

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an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a
5 microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet,
10 an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting ibuprofen from degradation
15 comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering ibuprofen to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, ibuprofen is released from the composition by
20 an enzyme-catalyzed release. In another preferred embodiment, ibuprofen is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and ibuprofen is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, ibuprofen is released
25 from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, ibuprofen is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is

controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching ibuprofen to a side chain of an amino acid to form an active agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, ibuprofen and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

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DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize ibuprofen and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of ibuprofen.

5 Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

The composition of the invention comprises ibuprofen covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one
10 of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have
15 primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains
20 constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino
25 acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

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The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be

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enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will
5 ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of
10 the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a
15 synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level
20 of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond
25 between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate

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weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border
 5 membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

10

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For
 15 example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could
 20 conceivably have a loading of 58%, although this may not be entirely practical.

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The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

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The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, ibuprofen is covalently attached to the polypeptide via the carboxylic acid group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

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There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized
5 adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions,
10 sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is
15 particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

20 Preferably, the resultant peptide-ibuprofen conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

25 An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product

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precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C.

- 5 The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

- 10 In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product
15 is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

- Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include
20 dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

- There have been over 30 different γ -alkyl glutamates prepared any one of which
25 may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for

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several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

- The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C.
- 5 The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

- 10 γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

- 15 γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

- Although illustrated and described above with reference to specific embodiments,
- 20 the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

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CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 ibuprofen covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein ibuprofen is covalently attached to a side
chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

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12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein ibuprofen is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing ibuprofen from said composition in a pH-dependent manner.

15 19. A method for protecting ibuprofen from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of ibuprofen from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching ibuprofen to said polypeptide.

20 21. A method for delivering ibuprofen to a patient comprising administering to said patient a composition comprising:

 a polypeptide; and

 ibuprofen covalently attached to said polypeptide.

25 22. The method of claim 21 wherein ibuprofen is released from said composition by an enzyme-catalyzed release.

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23. The method of claim 21 wherein ibuprofen is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and ibuprofen covalently attached to the polypeptide. Also provided is a method for delivery of ibuprofen to a patient comprising administering to the patient a composition comprising a polypeptide and ibuprofen covalently attached to the polypeptide. Also provided is a method for protecting
5 ibuprofen from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of ibuprofen from a composition comprising covalently attaching it to the polypeptide.

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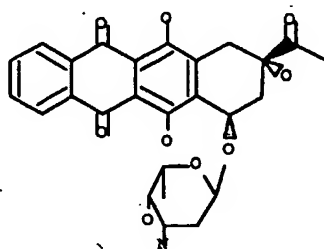
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING IDARUBICIN AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to idarubicin, as well as methods for protecting and administering idarubicin. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

Idarubicin is a known pharmaceutical agent that is used in the treatment of cancer.
15 Its chemical name is (7S,9S)-9-acetyl-7-[(3-amino-2,3,6-trideoxy- α -L-lyxo-hexopyranosyl)oxy]-7,8,9,10-tetrahydro-6,9,11-trihydroxy-5,12-naphthacenedioone. Its structure is:



The novel pharmaceutical compound of the present invention is useful in
20 accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical
25 agent, an adjuvant, or an inhibitor.

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Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase
5 markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly
10 acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the
15 active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and
20 aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can
25 also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage

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reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in

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the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (idarubicin) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching idarubicin to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising idarubicin microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and idarubicin covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Idarubicin preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a

carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting idarubicin from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering idarubicin to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, idarubicin is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, idarubicin is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and idarubicin is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, idarubicin is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, idarubicin is released from the composition in a sustained release.

In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

5 The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

(a) attaching idarubicin to a side chain of an amino acid to form an active agent/amino acid complex;

10 (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and

(c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

15 In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, idarubicin and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular
20 transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the
25 glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is

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described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

5 The present invention provides several benefits for active agent delivery. First, the invention can stabilize idarubicin and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of idarubicin. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

10 Idarubicin is the subject of GB 1467383 (1977), herein incorporated by reference, which describes how to make that drug.

 The composition of the invention comprises idarubicin covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more
15 naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

 Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the
20 local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

25 Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino

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acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

5 The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with
10 protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal
15 packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

 Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released.
20 The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is
25 often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

 Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's

decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other
5 hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine,
10 lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is
15 important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

20 As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the
25 kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of

active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

15

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent

delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application; for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

5 The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an
10 amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example
15 above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

20 The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal
25 enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using
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any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart
5 specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these
10 polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the
15 alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-
20 dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, idarubicin is covalently attached to the polypeptide via the hydroxyl group.

The polypeptide carrier can be prepared using conventional techniques. A
25 preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

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The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-idarubicin conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

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Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

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Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5 several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

 γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10 followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15 the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20 a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
25 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

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CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 idarubicin covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein idarubicin is covalently attached to a side
chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

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12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein idarubicin is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing idarubicin from said composition in a pH-dependent manner.

15 19. A method for protecting idarubicin from degradation comprising covalently attaching said active agent to a polypeptide.

20 20. A method for controlling release of idarubicin from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching idarubicin to said polypeptide.

21. A method for delivering idarubicin to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
idarubicin covalently attached to said polypeptide.

22. The method of claim 21 wherein idarubicin is released from said composition
25 by an enzyme-catalyzed release.

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23. The method of claim 21 wherein idarubicin is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

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Abstract

A composition comprising a polypeptide and idarubicin covalently attached to the polypeptide. Also provided is a method for delivery of idarubicin to a patient comprising administering to the patient a composition comprising a polypeptide and idarubicin covalently attached to the polypeptide. Also provided is a method for protecting idarubicin from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of idarubicin from a composition comprising covalently attaching it to the polypeptide.

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A NOVEL PHARMACEUTICAL COMPOUND CONTAINING ILODECAKIN AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to ilodecakin, as well as methods for protecting and administering ilodecakin. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

 Ilodecakin is a known pharmaceutical agent that is used in the treatment of
15 hepatitis, autoimmune disorders and HIV infections. Its chemical name is interleukin 10, and it is both isolatable from natural sources and capable of being synthesized by those of skill in the art.

 The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability
20 of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

25 Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another

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invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is

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unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

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SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (ilodecakin) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching ilodecakin to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising ilodecakin microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and ilodecakin covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Ilodecakin preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

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The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting ilodecakin from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering ilodecakin to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, ilodecakin is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, ilodecakin is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and ilodecakin is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, ilodecakin is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, ilodecakin is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

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The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching ilodecakin to a side chain of an amino acid to form an active agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, ilodecakin and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize ilodecakin and prevent its digestion in the stomach. In

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addition, the pharmacologic effect can be prolonged by delayed release of ilodecakin. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

5 The composition of the invention comprises ilodecakin covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or
10 more naturally occurring amino acids and one or more synthetic amino acids.

 Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the
15 conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

 Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding
20 are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best
25 model for determining forces contributing to protein stability is the solid reference state.

 The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior

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and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are
5 "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

10 Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only
15 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

20 Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For
25 instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will

A NOVEL PHARMACEUTICAL COMPOUND CONTAINING IMIGLUCERASE AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to imiglucerase, as well as methods for protecting and administering imiglucerase. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

 Imiglucerase is a known pharmaceutical agent that is used in the treatment of
15 Gaucher disease. Its chemical name is glucosyl-(human placenta isoenzyme protein moiety) 495-L-histidine-ceramidase. It is a recombinant glucocerebrosidase enzyme.

 The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered
20 product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

 Active agent delivery systems are often critical for the effective delivery of a
25 biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf

life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is

unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (imiglucerase) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching imiglucerase to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising imiglucerase microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and imiglucerase covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Imiglucerase preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting imiglucerase from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering imiglucerase to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, imiglucerase is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, imiglucerase is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and imiglucerase is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, imiglucerase is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, imiglucerase is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- 5 (a) attaching imiglucerase to a side chain of an amino acid to form an active agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

10 In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, imiglucerase and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and
15 wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a
20 carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention.
25 The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First,
30 the invention can stabilize imiglucerase and prevent its digestion in the stomach. In

addition, the pharmacologic effect can be prolonged by delayed release of imiglucerase. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

5 Imiglucerase is the subject of EP 401362 B (1996), based on priority application US 289589 (1988), herein incorporated by reference, which describes how to make that drug.

The composition of the invention comprises imiglucerase covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one
10 of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have
15 primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains
20 constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino
25 acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be

enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will
5 ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of
10 the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a
15 synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level
20 of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond
25 between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate

weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border
 5 membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

10

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For
 15 example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could
 20 conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, imiglucerase is covalently attached to the polypeptide via a peptide bond.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized
5 adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions,
10 sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is
15 particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

20 Preferably, the resultant peptide-imiglucerase conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

25 An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product

precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C.

- 5 The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

- 10 In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product
15 is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

- Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include
20 dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

- There have been over 30 different γ -alkyl glutamates prepared any one of which
25 may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for

several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

- The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C.
- 5 The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

- 10 γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

- 15 γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

- Although illustrated and described above with reference to specific embodiments,
- 20 the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 imiglucerase covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein imiglucerase is covalently attached to a
side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein imiglucerase is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing imiglucerase from said composition in a pH-dependent manner.

15 19. A method for protecting imiglucerase from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of imiglucerase from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching imiglucerase to said polypeptide.

20 21. A method for delivering imiglucerase to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
imiglucerase covalently attached to said polypeptide.

25 22. The method of claim 21 wherein imiglucerase is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein imiglucerase is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

- 5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and imiglucerase covalently attached to the polypeptide. Also provided is a method for delivery of imiglucerase to a patient comprising administering to the patient a composition comprising a polypeptide and
5 imiglucerase covalently attached to the polypeptide. Also provided is a method for protecting imiglucerase from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of imiglucerase from a composition comprising covalently attaching it to the polypeptide.

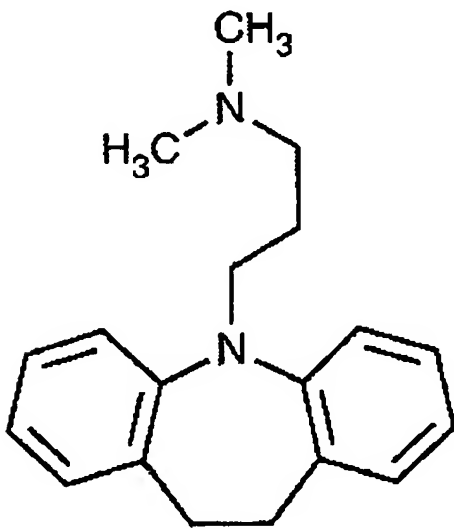
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING IMIPRAMINE AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to imipramine, as well as methods for protecting and administering imipramine. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

15 Imipramine is a known pharmaceutical agent that is used in the treatment of depression. Its structure is:



The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered

product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

5 Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another
10 invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of
15 cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme
20 degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example,
25 copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

10 SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (imipramine) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching imipramine to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising imipramine microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and imipramine covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a

heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Imipramine preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a
5 carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-
10 terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant
15 preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the
20 composition in a pH-dependent manner.

The invention also provides a method for protecting imipramine from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering imipramine to a patient, the patient being a human or a non-human animal, comprising administering to the patient a
25 composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, imipramine is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, imipramine is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed

release. In another preferred embodiment, the composition further comprises a microencapsulating agent and imipramine is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, imipramine is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, imipramine is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching imipramine to a side chain of an amino acid to form an active agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, imipramine and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the

glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention.

- 5 The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

- 10 The present invention provides several benefits for active agent delivery. First, the invention can stabilize imipramine and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of imipramine. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

- 15 The composition of the invention comprises imipramine covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or
20 more naturally occurring amino acids and one or more synthetic amino acids.

- Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the
25 conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes.

Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the

kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond
 5 between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and
 10 drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be
 15 controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the
 20 stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's

molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active

agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is

5 polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action

10 for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is

15 distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or

20 the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

25 In the present invention, the active agent is covalently attached to the polypeptide via a linker. This linker may be a small molecule containing 2-6 carbons and one or more functional groups (such as amines, amides, alcohols, or acids) or may be made up of a short chain of either amino acids or carbohydrates.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

5 The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex,
10 PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized
15 adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions,
20 sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is
25 particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-imipramine conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

5 **Acid/N-terminus conjugation**

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated

solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

5 **Preparation of γ -Alkyl Glutamate**

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered,
10 dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for
15 several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes
20 homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically
25 overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 imipramine covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein imipramine is covalently attached to a
side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein imipramine is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing imipramine from said composition in a pH-dependent manner.

15 19. A method for protecting imipramine from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of imipramine from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching imipramine to said polypeptide.

20 21. A method for delivering imipramine to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
imipramine covalently attached to said polypeptide.

25 22. The method of claim 21 wherein imipramine is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein imipramine is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

- 5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and imipramine covalently attached to the polypeptide. Also provided is a method for delivery of imipramine to a patient
5 comprising administering to the patient a composition comprising a polypeptide and imipramine covalently attached to the polypeptide. Also provided is a method for protecting imipramine from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of imipramine from a composition comprising covalently attaching it to the polypeptide.

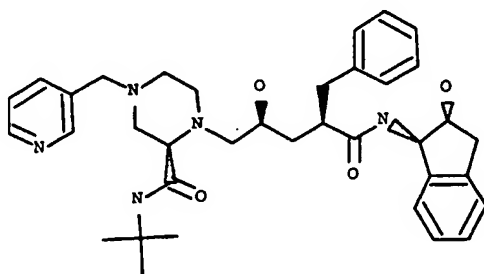
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING INDINAVIR AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to indinavir, as well as methods for protecting and administering indinavir. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

Indinavir is a known pharmaceutical agent that is used in the treatment of HIV
15 infection. Its chemical name is 2,3,5-trideoxy-N-[(1S,2R)-2,3-dihydro-2-hydroxy-1H-inden-1-yl]-5-[(2S)-2-[(1,1-dimethylethyl)amino]carbonyl]-4-(3-pyridinylmethyl)-1-piperazinyl]-2-(phenylmethyl)-D-erythro-pentonamide. Its structure is:



20 The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical

compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several

shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

10 In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral
15 administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group.
20 This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the
25 active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle
30 size of the active agent delivery system. Variable molecular weights have unpredictable

diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients.

- 5 Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

- The present invention provides covalent attachment of the active agent (indinavir)
10 to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching indinavir to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection.
15 In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

- 20 Alternatively, the present invention provides a pharmaceutical composition comprising indinavir microencapsulated by a polypeptide.

- The invention provides a composition comprising a polypeptide and indinavir covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a
25 heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Indinavir preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting indinavir from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering indinavir to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, indinavir is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, indinavir is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and indinavir is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, indinavir is released

from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, indinavir is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is
5 controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- 10 (a) attaching indinavir to a side chain of an amino acid to form an active agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride
15 (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, indinavir and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released
20 from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side
25 chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the
30 following detailed description are exemplary, but are not restrictive, of the invention.

The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

5 The present invention provides several benefits for active agent delivery. First, the invention can stabilize indinavir and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of indinavir. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted
10 delivery of active agents to specific sites of action.

Indinavir is the subject of EP 541168 B (1998), based on priority application US 789508 (1991), and U.S. Patent Number 5,413,999, herein incorporated by reference, which describes how to make that drug.

15 The composition of the invention comprises indinavir covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

20 Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The
25 folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding

are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded
5 protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect
10 refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are
15 "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

20 Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only
25 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

5 Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding.

10 Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all

15 be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple

20 active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain

25 length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the

carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a
5 macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several
10 aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of
15 lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's
20 molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular

weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could
5 conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-
10 terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the
15 preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the
20 peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid
25 moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively,
30 the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-

carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, indinavir is covalently attached to the polypeptide via the amine groups.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-indinavir conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5 several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10 followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15 the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20 a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
25 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 indinavir covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein indinavir is covalently attached to a side
chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein indinavir is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing indinavir from said composition in a pH-dependent manner.

15 19. A method for protecting indinavir from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of indinavir from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching indinavir to said polypeptide.

20 21. A method for delivering indinavir to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
indinavir covalently attached to said polypeptide.

25 22. The method of claim 21 wherein indinavir is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein indinavir is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

- 5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and indinavir covalently attached to the polypeptide. Also provided is a method for delivery of indinavir to a patient comprising administering to the patient a composition comprising a polypeptide and indinavir covalently attached to the polypeptide. Also provided is a method for protecting
5 indinavir from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of indinavir from a composition comprising covalently attaching it to the polypeptide.

A NOVEL PHARMACEUTICAL COMPOUND CONTAINING INFlixIMAB AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to infliximab, as well as methods for protecting and administering infliximab. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

 Infliximab is a known pharmaceutical agent that is used in the treatment of
15 arthritis and HIV infection. It is a monoclonal antibody targeting tumor necrosis factor alpha. Its chemical name is immunoglobulin G, anti-(human tumour necrosis factor) (human-mouse monoclonal cA2 heavy chain), disulfide with human-mouse monoclonal cA2 light chain, dimer.

 The novel pharmaceutical compound of the present invention is useful in
20 accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical
25 agent, an adjuvant, or an inhibitor.

 Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase

markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

5 Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres,
10 liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

 Active agent delivery systems also provide the ability to control the release of the
15 active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified
20 amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

 Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the
25 microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some

technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR

application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (infiximab) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching infiximab to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising infiximab microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and infiximab covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Infiximab preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is

an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a
5 microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet,
10 an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting infliximab from degradation
15 comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering infliximab to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, infliximab is released from the composition by
20 an enzyme-catalyzed release. In another preferred embodiment, infliximab is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and infliximab is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, infliximab is released
25 from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, infliximab is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is

controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method
5 comprises the steps of:

(a) attaching infliximab to a side chain of an amino acid to form an active agent/amino acid complex;

(b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and

10 (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, infliximab and a second active agent can be copolymerized in step (c). In another
15 preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine,
20 cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

25 It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize infliximab and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of infliximab.

- 5 Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

Infliximab is the subject of U.S. Patent Number yyyyyy, herein incorporated by reference, which describes how to make that drug.

- 10 The composition of the invention comprises infliximab covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or
15 more naturally occurring amino acids and one or more synthetic amino acids.

- Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the
20 conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

- Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding
25 are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded

protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational
5 entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant
10 force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has
15 maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is
20 a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious
25 chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

5 Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

10 Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

15 Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

20 As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of
25 active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant

groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropyl)glutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence,

maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action
5 for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is
10 distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or
15 the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

20 In the present invention, infliximab is covalently attached to the polypeptide via a peptide bond.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer
25 can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the

invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-infliximab conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5 several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10 followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15 the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20 a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
25 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 infliximab covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein infliximab is covalently attached to a side
chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein infliximab is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing infliximab from said composition in a pH-dependent manner.

15 19. A method for protecting infliximab from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of infliximab from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching infliximab to said polypeptide.

20 21. A method for delivering infliximab to a patient comprising administering to said patient a composition comprising:

 a polypeptide; and

 infliximab covalently attached to said polypeptide.

25 22. The method of claim 21 wherein infliximab is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein infliximab is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and infliximab covalently attached to the polypeptide. Also provided is a method for delivery of infliximab to a patient comprising administering to the patient a composition comprising a polypeptide and infliximab covalently attached to the polypeptide. Also provided is a method for protecting
5 infliximab from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of infliximab from a composition comprising covalently attaching it to the polypeptide.

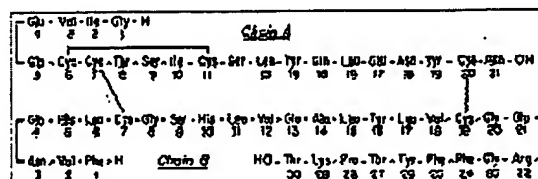
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING HUMAN INSULIN AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to human insulin, as well as methods for protecting and administering human insulin. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

Human insulin is a known pharmaceutical agent that is used in the treatment of
15 diabetes. Insulin human is a biosynthetic or semisynthetic protein that is structurally identical to endogenous insulin secreted by the beta cells of the human pancreas. Although structurally identical to endogenous human insulin, commercially available insulin human is *not* extracted from the human pancreas, but is prepared biosynthetically from cultures of genetically modified *Escherichia coli* or *Saccharomyces cerevisiae* or
20 semisynthetically by transpeptidation of pork insulin. Its structure is:



25 The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical

compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several

shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable

diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients.

- 5 Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

- The present invention provides covalent attachment of the active agent (human
10 insulin) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching human insulin to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through
15 conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

- 20 Alternatively, the present invention provides a pharmaceutical composition comprising human insulin microencapsulated by a polypeptide.

- The invention provides a composition comprising a polypeptide and human insulin covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids,
25 (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Human insulin preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting human insulin from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering human insulin to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, human insulin is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, human insulin is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and human insulin is released from the composition by dissolution of the microencapsulating agent. In another preferred

embodiment, human insulin is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, human insulin is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching human insulin to a side chain of an amino acid to form an active agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, human insulin and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize human insulin and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of human insulin. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

Human insulin is the subject of U.S. Patent Numbers 5,474,978 and 5,514,646, herein incorporated by reference, which describes how to make that drug.

The composition of the invention comprises human insulin covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes.

Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's
5 decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other
10 hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

15 Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

20 Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have
25 profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the

kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond
 5 between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and
 10 drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be
 15 controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the
 20 stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's

molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active

agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is
5 polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action
10 for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is
15 distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or
20 the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

25 In the present invention, human insulin is covalently attached to the polypeptide via a peptide bond.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides.

Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-human insulin conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

10 Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-

hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which
5 may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

10 The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

15 Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

20 Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

25 Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,

various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 human insulin covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein human insulin is covalently attached to a
side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein human insulin is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing human insulin from said composition in a pH-dependent manner.

15 19. A method for protecting human insulin from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of human insulin from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching human insulin to said polypeptide.

20 21. A method for delivering human insulin to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
human insulin covalently attached to said polypeptide.

25 22. The method of claim 21 wherein human insulin is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein human insulin is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and human insulin covalently attached to the polypeptide. Also provided is a method for delivery of human insulin to a patient comprising administering to the patient a composition comprising a polypeptide and human insulin covalently attached to the polypeptide. Also provided is a method for protecting human insulin from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of human insulin from a composition comprising covalently attaching it to the polypeptide.

A NOVEL PHARMACEUTICAL COMPOUND CONTAINING INTERFERON ALFACON-1 AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to interferon alfacon-1, as well as methods for protecting and administering interferon alfacon-1. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a
10 known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

 Interferon alfacon-1 is a known pharmaceutical agent that is used in the treatment
15 of viral infection and cancer. Its chemical name is interferon alpha 1 (human lymphoblast reduced), N-L-methionyl-22-L-arg-76-L-ala-78-L-asn-79-L-glu-86-L-tyr-90-L-tyr-156-L-thr-157-L-asn-158-L-leu.

 The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability
20 of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

25 Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another

invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is

unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (interferon alfacon-1) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching interferon alfacon-1 to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising interferon alfacon-1 microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and interferon alfacon-1 covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Interferon alfacon-1 preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant
5 preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the
10 composition in a pH-dependent manner.

The invention also provides a method for protecting interferon alfacon-1 from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering interferon alfacon-1 to a patient, the patient being a human or a non-human animal, comprising administering to
15 the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, interferon alfacon-1 is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, interferon alfacon-1 is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the
20 composition further comprises a microencapsulating agent and interferon alfacon-1 is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, interferon alfacon-1 is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, interferon alfacon-1 is released from the composition in a sustained release. In yet another
25 preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- 5 (a) attaching interferon alfacon-1 to a side chain of an amino acid to form an active agent/amino acid complex;
 - (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
 - (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).
- 10 In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, interferon alfacon-1 and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and
- 15 wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a
- 20 carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention.

- 25 The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

- 30 The present invention provides several benefits for active agent delivery. First, the invention can stabilize interferon alfacon-1 and prevent its digestion in the stomach.

In addition, the pharmacologic effect can be prolonged by delayed release of interferon alfacon-1. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

5 Interferon alfacon-1 is the subject of EP 422697 B (1994), based on priority US application 375494 (1982), herein incorporated by reference, which describes how to make that drug.

The composition of the invention comprises interferon alfacon-1 covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a
10 homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have
15 primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains
20 constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino
25 acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be

enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will
5 ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of
10 the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a
15 synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level
20 of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond
25 between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate

weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border
5 membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

10

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For
15 example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could
20 conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, interferon alfacon-1 is covalently attached to the polypeptide via a peptide bond.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized
5 adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions,
10 sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is
15 particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

20 Preferably, the resultant peptide-interferon alfacon-1 conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

25 An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product

precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C.

- 5 The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

- 10 In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product
15 is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

- Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include
20 dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

- There have been over 30 different γ -alkyl glutamates prepared any one of which
25 may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for

several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C.

- 5 The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

- 10 γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

- 15 γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

- 20 Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 interferon alfacon-1 covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein interferon alfacon-1 is covalently attached
to a side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein interferon alfacon-1 is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing interferon alfacon-1 from said composition in a pH-dependent manner.

15 19. A method for protecting interferon alfacon-1 from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of interferon alfacon-1 from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching interferon alfacon-1 to said polypeptide.

20 21. A method for delivering interferon alfacon-1 to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
interferon alfacon-1 covalently attached to said polypeptide.

25 22. The method of claim 21 wherein interferon alfacon-1 is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein interferon alfacon-1 is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and interferon alfacon-1 covalently attached to the polypeptide. Also provided is a method for delivery of interferon alfacon-1 to a patient comprising administering to the patient a composition comprising a polypeptide and interferon alfacon-1 covalently attached to the polypeptide. Also provided is a method for protecting interferon alfacon-1 from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of interferon alfacon-1 from a composition comprising covalently attaching it to the polypeptide.

A NOVEL PHARMACEUTICAL COMPOUND CONTAINING INTERFERON BETA-1A AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to interferon beta-1a, as well as methods for protecting and administering interferon beta-1a. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a
10 known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

Interferon beta-1a is a known pharmaceutical agent that is used in the treatment of
15 multiple sclerosis, viral infection and cancer. It is 145258-61-3 human fibroblast protein moiety 74899-73-3 pre-(human fibroblast protein moiety reduced) 74899-71-1 human fibroblast protein moiety reduced. Biogen was awarded European patent number 41313 for the production of interferon beta through recombinant technology. The patent covered recombinant DNA molecules, transformed hosts and methods for producing recombinant
20 interferon beta proteins.

The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type;
25 and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these

systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf
5 life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as
10 resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the
15 stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human
20 growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties
25 to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble

microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients.

Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

5 The present invention provides covalent attachment of the active agent (interferon beta-1a) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching interferon beta-1a to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the
10 polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This
15 enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising interferon beta-1a microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and interferon beta-1a covalently attached to the polypeptide. Preferably, the polypeptide is (i) an
20 oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

25 Interferon beta-1a preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to

the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

5 The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

10 Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

15 The invention also provides a method for protecting interferon beta-1a from degradation comprising covalently attaching it to a polypeptide.

 The invention also provides a method for delivering interferon beta-1a to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently
20 attached to the polypeptide. In a preferred embodiment, interferon beta-1a is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, interferon beta-1a is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and interferon beta-1a is released from the
25 composition by dissolution of the microencapsulating agent. In another preferred embodiment, interferon beta-1a is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, interferon beta-1a is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the

polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

(a) attaching interferon beta-1a to a side chain of an amino acid to form an active agent/amino acid complex;

(b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and

(c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, interferon beta-1a and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize interferon beta-1a and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of interferon
5 beta-1a. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

The composition of the invention comprises interferon beta-1a covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a
10 homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have
15 primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains
20 constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino
25 acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be

enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will
5 ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of
10 the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a
15 synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level
20 of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond
25 between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate

weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border
 5 membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

10

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For
 15 example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could
 20 conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, interferon beta-1a is covalently attached to the polypeptide via a peptide bond.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized
5 adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions,
10 sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is
15 particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

20 Preferably, the resultant peptide-interferon beta-1a conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

25 An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product

precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C.

- 5 The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

- 10 In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product
15 is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

- Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include
20 dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

- There have been over 30 different γ -alkyl glutamates prepared any one of which
25 may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for

several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

- The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C.
- 5 The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

- 10 γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

- 15 γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

- 20 Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 interferon beta-1a covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein interferon beta-1a is covalently attached
to a side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein interferon beta-1a is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing interferon beta-1a from said composition in a pH-dependent manner.

15 19. A method for protecting interferon beta-1a from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of interferon beta-1a from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching interferon beta-1a to said polypeptide.

20 21. A method for delivering interferon beta-1a to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
interferon beta-1a covalently attached to said polypeptide.

25 22. The method of claim 21 wherein interferon beta-1a is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein interferon beta-1a is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and interferon beta-1a covalently attached to the polypeptide. Also provided is a method for delivery of interferon beta-1a to a patient comprising administering to the patient a composition comprising a

5 polypeptide and interferon beta-1a covalently attached to the polypeptide. Also provided is a method for protecting interferon beta-1a from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of interferon beta-1a from a composition comprising covalently attaching it to the polypeptide.

A NOVEL PHARMACEUTICAL COMPOUND CONTAINING INTERLEUKIN-2 AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to interleukin-2, as well as methods for protecting and administering interleukin-2. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

 Interleukin-2 is a known pharmaceutical agent that is used in the treatment of
15 renal cell carcinoma. IL-2 promotes proliferation, differentiation, and recruitment of T and B cells, natural killer (NK) cells, and thymocytes; IL-2 also causes cytolytic activity in a subset of lymphocytes and subsequent interactions between the immune system and malignant cells; IL-2 can stimulate lymphokine-activated killer (LAK) cells and tumor-infiltrating lymphocytes (TIL) cells. LAK cells (which are derived from lymphocytes
20 from a patient and incubated in IL-2) have the ability to lyse cells which are resistant to NK cells.

 The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered
25 product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase
5 markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly
10 acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the
15 active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and
20 aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can
25 also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage

reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some
5 technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically
10 require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the
15 gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The
20 released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that
25 incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly
30 or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in

the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (interleukin-2) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching interleukin-2 to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising interleukin-2 microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and interleukin-2 covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Interleukin-2 preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a

carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting interleukin-2 from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering interleukin-2 to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, interleukin-2 is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, interleukin-2 is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and interleukin-2 is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, interleukin-2 is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, interleukin-2 is released from the

composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic
5 release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching interleukin-2 to a side chain of an amino acid to form an active
10 agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

15 In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, interleukin-2 and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and
20 wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a
25 carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention.
30 The general applications of this invention to other active pharmaceutical agents is

described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

5 The present invention provides several benefits for active agent delivery. First, the invention can stabilize interleukin-2 and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of interleukin-2. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

10 The composition of the invention comprises interleukin-2 covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or
15 more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the
20 conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding
25 are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded

protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational
5 entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular
10 bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has
15 maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is
20 a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious
25 chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

5 Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

10 Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

15 Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

20 As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of
25 active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant

groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence,

maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action
5 for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is
10 distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or
15 the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

20 In the present invention, interleukin-2 is covalently attached to the polypeptide via a peptide bond.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer
25 can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the

invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-interleukin-2 conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5 several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10 followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15 the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20 a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
25 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 interleukin-2 covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein interleukin-2 is covalently attached to a side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein interleukin-2 is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing interleukin-2 from said composition in a pH-dependent manner.

15 19. A method for protecting interleukin-2 from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of interleukin-2 from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching interleukin-2 to said polypeptide.

20 21. A method for delivering interleukin-2 to a patient comprising administering to said patient a composition comprising:
 a polypeptide; and
 interleukin-2 covalently attached to said polypeptide.

25 22. The method of claim 21 wherein interleukin-2 is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein interleukin-2 is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and interleukin-2 covalently attached to the polypeptide. Also provided is a method for delivery of interleukin-2 to a patient comprising administering to the patient a composition comprising a polypeptide and
5 interleukin-2 covalently attached to the polypeptide. Also provided is a method for protecting interleukin-2 from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of interleukin-2 from a composition comprising covalently attaching it to the polypeptide.

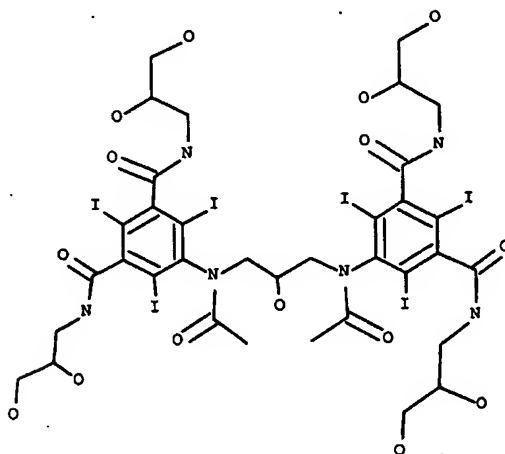
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING IODIXANOL AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to iodixanol, as well as methods for protecting and administering iodixanol. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

Iodixanol is a known pharmaceutical agent that is used as a contrast medium for
15 medical imaging. Its chemical name is 5,5'-[(2-hydroxy-1,3-propanediyl)bis(acetylamino)]bis[N,N'-bis(2,3-dihydroxypropyl)-2,4,6-triodo-1,3-benzenedicarboxamide]. Its structure is:



The novel pharmaceutical compound of the present invention is useful in
20 accomplishing one or more of the following goals: enhancement of the chemical stability

of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified

amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that

incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (iodixanol) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching iodixanol to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising iodixanol microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and iodixanol covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a

heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

5 Iodixanol preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is
10 an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The
15 microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be
20 conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting iodixanol from degradation comprising covalently attaching it to a polypeptide.

25 The invention also provides a method for delivering iodixanol to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, iodixanol is released from the composition by

an enzyme-catalyzed release. In another preferred embodiment, iodixanol is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and iodixanol is released from the composition by dissolution
5 of the microencapsulating agent. In another preferred embodiment, iodixanol is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, iodixanol is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is
10 controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- 15 (a) attaching iodixanol to a side chain of an amino acid to form an active agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride
20 (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, iodixanol and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released
25 from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side
30 chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the

glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention.

- 5 The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

- 10 The present invention provides several benefits for active agent delivery. First, the invention can stabilize iodixanol and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of iodixanol. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

- 15 Iodixanol is the subject of U.S. Patent Number 5,349,085, herein incorporated by reference, which describes how to make that drug.

- The composition of the invention comprises iodixanol covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more
20 naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

- Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the
25 local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The

folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and

at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

5 Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

10 Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

15 Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

20 Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

25 Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain

length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to

poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

5 The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal
10 enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using
15 any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart
20 specific properties to the drug delivery system.

 The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these
25 polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the
30 alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, iodixanol is covalently attached to the polypeptide via the hydroxyl group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the

mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-iodixanol conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then
5 added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated
10 solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

15 There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

20 γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product
25 precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product,
5 which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and
10 filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 iodixanol covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein iodixanol is covalently attached to a side
chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein iodixanol is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing iodixanol from said composition in a pH-dependent manner.

15 19. A method for protecting iodixanol from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of iodixanol from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching iodixanol to said polypeptide.

20 21. A method for delivering iodixanol to a patient comprising administering to said patient a composition comprising:
 a polypeptide; and
 iodixanol covalently attached to said polypeptide.

25 22. The method of claim 21 wherein iodixanol is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein iodixanol is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

10 Abstract

A composition comprising a polypeptide and iodixanol covalently attached to the polypeptide. Also provided is a method for delivery of iodixanol to a patient comprising administering to the patient a composition comprising a polypeptide and iodixanol covalently attached to the polypeptide. Also provided is a method for protecting
15 iodixanol from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of iodixanol from a composition comprising covalently attaching it to the polypeptide.

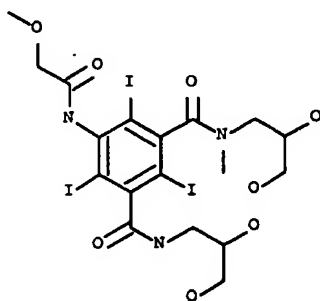
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING IOPROMIDE AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to iopromide, as well as methods for protecting and administering iopromide. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

 Iopromide is a known pharmaceutical agent that is used as an X-ray contrast
15 medium. Its chemical name is N,N'-bis(2,3-dihydroxypropyl)-2,4,6-triiodo-5-[(2-methoxyacetyl)amino]-N-methyl-1,3-benzenedicarboxamide. Its structure is:



 The novel pharmaceutical compound of the present invention is useful in
accomplishing one or more of the following goals: enhancement of the chemical stability
20 of the original compound; alteration of the release profile of an orally administered
product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type;
and provision for an oral dosage form when none exists. The novel pharmaceutical

compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several

shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

10 In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral
15 administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group.
20 This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the
25 active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle
30 size of the active agent delivery system. Variable molecular weights have unpredictable

diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients.

- 5 Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent
10 (iopromide) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching iopromide to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through
15 conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

- 20 Alternatively, the present invention provides a pharmaceutical composition comprising iopromide microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and iopromide covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a
25 heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Iopromide preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting iopromide from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering iopromide to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, iopromide is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, iopromide is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and iopromide is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, iopromide is released

from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, iopromide is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is
5 controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- 10 (a) attaching iopromide to a side chain of an amino acid to form an active agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride
15 (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, iopromide and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released
20 from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side
25 chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the
30 following detailed description are exemplary, but are not restrictive, of the invention.

The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

5 The present invention provides several benefits for active agent delivery. First, the invention can stabilize iopromide and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of iopromide. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also
10 allows targeted delivery of active agents to specific sites of action.

Iopromide is the subject of U.S. Patent Number 4,364,921, herein incorporated by reference, which describes how to make that drug.

The composition of the invention comprises iopromide covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one
15 of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have
20 primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains
25 constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a

particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best
5 model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with
10 protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and
15 contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a
20 peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and
25 at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

5 Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

 Ionizing amino acids can be selected for pH controlled peptide unfolding.
10 Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

 Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all
15 be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

 Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple
20 active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

 As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain
25 length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the

carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a
 5 macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several
 10 aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of
 15 lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's
 20 molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular

weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could
5 conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-
10 terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the
15 preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the
20 peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid
25 moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively,
30 the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-

carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, iopromide is covalently attached to the polypeptide via the hydroxyl group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-iodomide conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5 several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10 followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15 the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20 a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
25 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 iopromide covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein iopromide is covalently attached to a side
chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein iopromide is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing iopromide from said composition in a pH-dependent manner.

15 19. A method for protecting iopromide from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of iopromide from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching iopromide to said polypeptide.

20 21. A method for delivering iopromide to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
iopromide covalently attached to said polypeptide.

25 22. The method of claim 21 wherein iopromide is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein iopromide is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

- 5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and iopromide covalently attached to the polypeptide. Also provided is a method for delivery of iopromide to a patient comprising administering to the patient a composition comprising a polypeptide and iopromide covalently attached to the polypeptide. Also provided is a method for protecting
5 iopromide from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of iopromide from a composition comprising covalently attaching it to the polypeptide.

A NOVEL PHARMACEUTICAL COMPOUND CONTAINING IOXAGLATE AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to ioxaglate, as well as methods for protecting and administering ioxaglate. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

Ioxaglate is a known pharmaceutical agent that is used as a radiopaque contrast
15 aide. It is usually used as a combination of Ioxaglate meglumine and Ioxaglate sodium. Both units can be attached to a polypeptide carrier.

The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered
20 product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a
25 biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf

life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is

unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically
5 require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous
10 pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The
15 released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that
20 incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable
25 diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited
30 to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (ioxaglate) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching ioxaglate to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising ioxaglate microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and ioxaglate covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Ioxaglate preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting ioxaglate from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering ioxaglate to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, ioxaglate is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, ioxaglate is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and ioxaglate is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, ioxaglate is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, ioxaglate is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- 5 (a) attaching ioxaglate to a side chain of an amino acid to form an active agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

- 10 In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, ioxaglate and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the
- 15 active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a
- 20 carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

- It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention.
- 25 The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

- The present invention provides several benefits for active agent delivery. First,
- 30 the invention can stabilize ioxaglate and prevent its digestion in the stomach. In addition,

the pharmacologic effect can be prolonged by delayed release of ioxaglate. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

5 The composition of the invention comprises ioxaglate covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or
10 more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the
15 conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding
20 are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best
25 model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior

and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are
5 "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

10 Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only
15 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

20 Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For
25 instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will

ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all
5 be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple
10 active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain
15 length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of
20 active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate
25 weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the

jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

5

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order

to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example
5 above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

10 The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal
15 enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-
20 carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart
25 specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these
30 polypeptides through a spacer or linker on the pendant group, which is terminated,

preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, ioxaglate is covalently attached to the polypeptide via the hydroxyl group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known

intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions,
5 sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is
10 particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

15 Preferably, the resultant peptide-ioxaglate conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

20 An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or
25 dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product
5 precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product,
10 which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and
15 filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 ioxaglate covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein ioxaglate is covalently attached to a side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein ioxaglate is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing ioxaglate from said composition in a pH-dependent manner.

15 19. A method for protecting ioxaglate from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of ioxaglate from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching ioxaglate to said polypeptide.

20 21. A method for delivering ioxaglate to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
ioxaglate covalently attached to said polypeptide.

25 22. The method of claim 21 wherein ioxaglate is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein ioxaglate is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and ioxaglate covalently attached to the polypeptide. Also provided is a method for delivery of ioxaglate to a patient comprising administering to the patient a composition comprising a polypeptide and ioxaglate covalently attached to the polypeptide. Also provided is a method for protecting ioxaglate from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of ioxaglate from a composition comprising covalently attaching it to the polypeptide.

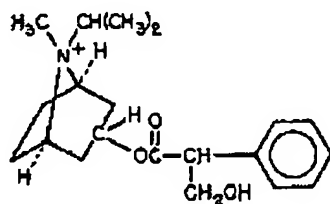
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING IPRATROPIUM AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to ipratropium, as well as methods for protecting and administering ipratropium. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

Ipratropium is used as a bronchodilator for the long-term symptomatic treatment
15 of reversible bronchospasm associated with chronic obstructive pulmonary disease (COPD). Its structure is:



The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability
20 of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase
5 markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly
10 acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the
15 active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and
20 aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can
25 also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage

reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in

the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (ipratropium) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching ipratropium to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising ipratropium microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and ipratropium covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Ipratropium preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a

carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting ipratropium from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering ipratropium to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, ipratropium is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, ipratropium is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and ipratropium is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, ipratropium is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, ipratropium is released from the

composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic
5 release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching ipratropium to a side chain of an amino acid to form an active
10 agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA)
from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride
(NCA).

15 In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, ipratropium and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the
20 active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a
25 carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention.
30 The general applications of this invention to other active pharmaceutical agents is

described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

5 The present invention provides several benefits for active agent delivery. First, the invention can stabilize ipratropium and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of ipratropium. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

10 The composition of the invention comprises ipratropium covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or
15 more naturally occurring amino acids and one or more synthetic amino acids.

 Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the
20 conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

 Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding
25 are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded

protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational
5 entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular
10 bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has
15 maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is
20 a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious
25 chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

5 Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

10 Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

15 Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

20 As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

25

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
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Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant

groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence,

maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action
5 for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is
10 distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or
15 the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

20 In the present invention, ipratropium is covalently attached to the polypeptide via the hydroxyl group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer
25 can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the

invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-ipratropium conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5 several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10 followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15 the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20 a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
25 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 ipratropium covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein ipratropium is covalently attached to a
side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein ipratropium is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing ipratropium from said composition in a pH-dependent manner.

15 19. A method for protecting ipratropium from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of ipratropium from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching ipratropium to said polypeptide.

20 21. A method for delivering ipratropium to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
ipratropium covalently attached to said polypeptide.

25 22. The method of claim 21 wherein ipratropium is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein ipratropium is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and ipratropium covalently attached to the polypeptide. Also provided is a method for delivery of ipratropium to a patient comprising administering to the patient a composition comprising a polypeptide and ipratropium covalently attached to the polypeptide. Also provided is a method for
5 protecting ipratropium from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of ipratropium from a composition comprising covalently attaching it to the polypeptide.

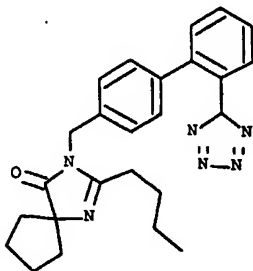
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING IRBESARTAN AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to irbesartan, as well as methods for protecting and administering irbesartan. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

 Irbesartan is a known pharmaceutical agent that is used in the treatment of
15 hypertension. Its chemical name is 2-butyl-3-[[[2'-(1H-tetrazol-5-yl)[1,1'-biphenyl]-4-yl]methyl]-1,3-diazaspiro[4.4]non-1-en-4-one. Its structure is:



 The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability
20 of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase
5 markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly
10 acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the
15 active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and
20 aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can
25 also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage

reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in

the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (irbesartan) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching irbesartan to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising irbesartan microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and irbesartan covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Irbesartan preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a

carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting irbesartan from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering irbesartan to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, irbesartan is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, irbesartan is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and irbesartan is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, irbesartan is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, irbesartan is released from the composition in a sustained release.

In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

5 The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching irbesartan to a side chain of an amino acid to form an active agent/amino acid complex;
- 10 (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

 In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a
15 second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, irbesartan and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular
20 transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the
25 glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

 It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is

described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

5 The present invention provides several benefits for active agent delivery. First, the invention can stabilize irbesartan and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of irbesartan. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

10 Irbesartan is the subject of U.S. Patent Number 5,270,317, herein incorporated by reference, which describes how to make that drug.

15 The composition of the invention comprises irbesartan covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

20 Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

25 Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino

acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

5 The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with
10 protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are “pushed out” of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal
15 packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

 Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released.
20 The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is
25 often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

 Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's

decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other
5 hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine,
10 lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is
15 important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

20 As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the
25 kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of

active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery.

- 5 Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border
- 10 membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

15

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

- Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For
- 20 example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent

delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

5 The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an
10 amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example
15 above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

20 The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal
25 enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using
30

any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart
5 specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these
10 polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the
15 alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-
20 dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, the active agent is covalently attached to the polypeptide via a linker. This linker may be a small molecule containing 2-6 carbons and one or more functional groups (such as amines, amides, alcohols, or acids) or may be made up of a
25 short chain of either amino acids or carbohydrates.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-irbesartan conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

CW232P

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

 γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 irbesartan covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more synthetic amino acids.
7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
15 one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein irbesartan is covalently attached to a side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
10. The composition of claim 9 wherein said microencapsulating agent is
20 selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein irbesartan is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing irbesartan from said composition in a pH-dependent manner.

15 19. A method for protecting irbesartan from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of irbesartan from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching irbesartan to said polypeptide.

20 21. A method for delivering irbesartan to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
irbesartan covalently attached to said polypeptide.

25 22. The method of claim 21 wherein irbesartan is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein irbesartan is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and irbesartan covalently attached to the polypeptide. Also provided is a method for delivery of irbesartan to a patient comprising administering to the patient a composition comprising a polypeptide and irbesartan covalently attached to the polypeptide. Also provided is a method for protecting irbesartan from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of irbesartan from a composition comprising covalently attaching it to the polypeptide.

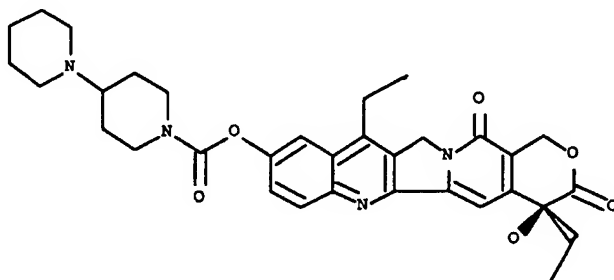
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING IRINOTECAN AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to irinotecan, as well as methods for protecting and administering irinotecan. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

Irinotecan is a known pharmaceutical agent that is used in the treatment of cancer.
15 Its chemical name is [1,4'-bipiperidine]-1'-carboxylic acid (S)-4,11-diethyl-3,4,12,14-tetrahydro-4-hydroxy-3,14-dioxo-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl ester. Its structure is:



20 The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical

compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several

shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

10 In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral
15 administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group.
20 This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the
25 active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle
30 size of the active agent delivery system. Variable molecular weights have unpredictable

diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients.

- 5 Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent
10 (irinotecan) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching irinotecan to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through
15 conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

- 20 Alternatively, the present invention provides a pharmaceutical composition comprising irinotecan microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and irinotecan covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a
25 heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Irinotecan preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting irinotecan from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering irinotecan to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, irinotecan is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, irinotecan is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and irinotecan is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, irinotecan is released

from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, irinotecan is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is
5 controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- 10 (a) attaching irinotecan to a side chain of an amino acid to form an active agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride
15 (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, irinotecan and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released
20 from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side
25 chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the
30 following detailed description are exemplary, but are not restrictive, of the invention.

The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

5 The present invention provides several benefits for active agent delivery. First, the invention can stabilize irinotecan and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of irinotecan. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also
10 allows targeted delivery of active agents to specific sites of action.

Irinotecan is the subject of U.S. Patent Number 4,604,463, herein incorporated by reference, which describes how to make that drug.

15 The composition of the invention comprises irinotecan covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

20 Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains
25 constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a

particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best
5 model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior
10 and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and
15 contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a
20 peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and
25 at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

- 5 Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding.

- 10 Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

- Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all
15 be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

- Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple
20 active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

- As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain
25 length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the

carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a
 5 macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several
 10 aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of
 15 lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's
 20 molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular

weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could
5 conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-
10 terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the
15 preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the
20 peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid
25 moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively,
30 the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-

carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, irinotecan is covalently attached to the polypeptide via the hydroxyl group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-irinotecan conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5 several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10 followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15 the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20 a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
25 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 irinotecan covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein irinotecan is covalently attached to a side
chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein irinotecan is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing irinotecan from said composition in a pH-dependent manner.

15 19. A method for protecting irinotecan from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of irinotecan from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching irinotecan to said polypeptide.

20 21. A method for delivering irinotecan to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
irinotecan covalently attached to said polypeptide.

25 22. The method of claim 21 wherein irinotecan is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein irinotecan is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

- 5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and irinotecan covalently attached to the polypeptide. Also provided is a method for delivery of irinotecan to a patient comprising administering to the patient a composition comprising a polypeptide and irinotecan covalently attached to the polypeptide. Also provided is a method for protecting
5 irinotecan from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of irinotecan from a composition comprising covalently attaching it to the polypeptide.

**A NOVEL PHARMACEUTICAL COMPOUND CONTAINING
ISOSORBIDE DINITRATE AND METHODS OF MAKING AND USING SAME**

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to isosorbide dinitrate, as well as methods for protecting and administering isosorbide dinitrate. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a
10 known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

15 Isosorbide dinitrate is a known pharmaceutical agent that is used in the treatment of angina. It is made up of the organic nitrates and nitrites are esters of nitrous or nitric acid, primarily amyl nitrite.

20 The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

25 Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf

life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is

unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (isosorbide dinitrate) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching isosorbide dinitrate to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising isosorbide dinitrate microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and isosorbide dinitrate covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Isosorbide dinitrate preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting isosorbide dinitrate from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering isosorbide dinitrate to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, isosorbide dinitrate is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, isosorbide dinitrate is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and isosorbide dinitrate is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, isosorbide dinitrate is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, isosorbide dinitrate is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

(a) attaching isosorbide dinitrate to a side chain of an amino acid to form an active agent/amino acid complex;

(b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and

(c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, isosorbide dinitrate and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize isosorbide dinitrate and prevent its digestion in the stomach.

In addition, the pharmacologic effect can be prolonged by delayed release of isosorbide dinitrate. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

5 The composition of the invention comprises isosorbide dinitrate covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of
10 one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the
15 conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding
20 are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best
25 model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior

and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are
5 "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

10 Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only
15 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

20 Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For
25 instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding.
Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will

ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the

jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

5

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order

to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example
5 above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

10 The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal
15 enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using
20 any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart
25 specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these
30 polypeptides through a spacer or linker on the pendant group, which is terminated,

preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, isosorbide dinitrate is covalently attached to the polypeptide via the nitrite group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known

intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-isosorbide dinitrate conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product
5 precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product,
10 which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and
15 filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 isosorbide dinitrate covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein isosorbide dinitrate is covalently attached
to a side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein isosorbide dinitrate is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing isosorbide dinitrate from said composition in a pH-dependent manner.

15 19. A method for protecting isosorbide dinitrate from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of isosorbide dinitrate from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching isosorbide dinitrate to said polypeptide.

20 21. A method for delivering isosorbide dinitrate to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
isosorbide dinitrate covalently attached to said polypeptide.

25 22. The method of claim 21 wherein isosorbide dinitrate is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein isosorbide dinitrate is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and isosorbide dinitrate covalently attached to the polypeptide. Also provided is a method for delivery of isosorbide dinitrate to a patient comprising administering to the patient a composition comprising a polypeptide and isosorbide dinitrate covalently attached to the polypeptide. Also provided is a method for protecting isosorbide dinitrate from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of isosorbide dinitrate from a composition comprising covalently attaching it to the polypeptide.

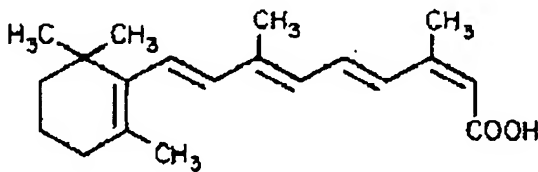
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING ISOTRETINOIN AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to isotretinoin, as well as methods for protecting and administering isotretinoin. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the
10 usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

 Isotretinoin is a known pharmaceutical agent that is used in the treatment of acne.
15 Its structure is:



 The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered
20 product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

 Active agent delivery systems are often critical for the effective delivery of a
25 biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken

under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even
5 reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of
10 cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the
15 active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release
20 through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several
25 shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent

in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

5 In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral
10 administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group.
15 This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the
20 active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

 It is also important to control the molecular weight, molecular size and particle
25 size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients.
30 Particle size not only becomes a problem with injectable drugs, as in the HAR

application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent
5 (isotretinoin) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching isotretinoin to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through
10 conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

15 Alternatively, the present invention provides a pharmaceutical composition comprising isotretinoin microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and isotretinoin covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a
20 heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Isotretinoin preferably is covalently attached to a side chain, the N-terminus or the
25 C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is

an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a
5 microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet,
10 an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting isotretinoin from degradation
15 comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering isotretinoin to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, isotretinoin is released from the composition by
20 an enzyme-catalyzed release. In another preferred embodiment, isotretinoin is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and isotretinoin is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, isotretinoin is
25 released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, isotretinoin is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be

microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method
5 comprises the steps of:

- (a) attaching isotretinoin to a side chain of an amino acid to form an active agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA)
from the active agent/amino acid complex; and
- 10 (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, isotretinoin and a second active agent can be copolymerized in step (c). In another
15 preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine,
20 cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

25 It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize isotretinoin and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of isotretinoin.

5 Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

The composition of the invention comprises isotretinoin covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one

10 of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have

15 primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains

20 constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino

25 acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be

enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will
5 ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of
10 the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a
15 synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level
20 of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond
25 between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate

weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border
 5 membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

10

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For
 15 example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could
 20 conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, isotretinoin is covalently attached to the polypeptide via the carboxylic acid group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized
5 adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions,
10 sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is
15 particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

20 Preferably, the resultant peptide-isotretinoin conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

25 An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product

precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

- The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C.
- 5 The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

- 10 In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product
- 15 is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

- Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include
- 20 dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

- There have been over 30 different γ -alkyl glutamates prepared any one of which
- 25 may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for

several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C.

- 5 The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

- 10 γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

- 15 γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

- 20 Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 isotretinoin covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein isotretinoin is covalently attached to a
side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein isotretinoin is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing isotretinoin from said composition in a pH-dependent manner.

15 19. A method for protecting isotretinoin from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of isotretinoin from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching isotretinoin to said polypeptide.

20 21. A method for delivering isotretinoin to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
isotretinoin covalently attached to said polypeptide.

25 22. The method of claim 21 wherein isotretinoin is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein isotretinoin is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

- 5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and isotretinoin covalently attached to the polypeptide. Also provided is a method for delivery of isotretinoin to a patient comprising administering to the patient a composition comprising a polypeptide and isotretinoin covalently attached to the polypeptide. Also provided is a method for protecting isotretinoin from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of isotretinoin from a composition comprising covalently attaching it to the polypeptide.

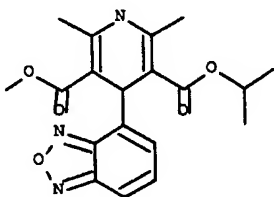
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING ISRADIPINE AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to isradipine, as well as methods for protecting and administering isradipine. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

 Isradipine is a known pharmaceutical agent that is used in the treatment of
15 hypertension. Its chemical name is 4-(4-benzofurazanyl)-1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylic acid methyl 1-methylethyl ester. Its structure is:



 The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability
20 of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase
5 markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly
10 acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the
15 active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and
20 aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can
25 also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage

reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in

the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (isradipine) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching isradipine to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising isradipine microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and isradipine covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Isradipine preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a

carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting isradipine from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering isradipine to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, isradipine is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, isradipine is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and isradipine is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, isradipine is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, isradipine is released from the composition in a sustained release.

In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

5 The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching isradipine to a side chain of an amino acid to form an active agent/amino acid complex;
- 10 (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

 In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a
15 second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, isradipine and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular
20 transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the
25 glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

 It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is

described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize isradipine and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of isradipine. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

Isradipine is the subject of EP 150 B (1981) and UK 2037766 B (1983), herein incorporated by reference, which describes how to make that drug.

The composition of the invention comprises isradipine covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a

particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

- 5 Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

- Ionizing amino acids can be selected for pH controlled peptide unfolding.
- 10 Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

- Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all
- 15 be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

- Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple
- 20 active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

- As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain
- 25 length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the

carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a
5 macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several
10 aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of
15 lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's
20 molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular

weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could
5 conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-
10 terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the
15 preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the
20 peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid
25 moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively,
30 the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-

carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart
5 specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine,
10 polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be
15 attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the
20 attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, the active agent is covalently attached to the polypeptide via a linker. This linker may be a small molecule containing 2-6 carbons and one or more
25 functional groups (such as amines, amides, alcohols, or acids) or may be made up of a short chain of either amino acids or carbohydrates.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides.

Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-isradipine conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

10 Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-

hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

5 There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

10 The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

15 Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

20 Preparation of Poly(γ -Alkyl Glutamate)

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

25 Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,

various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 isradipine covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein isradipine is covalently attached to a side
chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein isradipine is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing isradipine from said composition in a pH-dependent manner.

15 19. A method for protecting isradipine from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of isradipine from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching isradipine to said polypeptide.

20 21. A method for delivering isradipine to a patient comprising administering to said patient a composition comprising:
 a polypeptide; and
 isradipine covalently attached to said polypeptide.

25 22. The method of claim 21 wherein isradipine is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein isradipine is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

10

Abstract

A composition comprising a polypeptide and isradipine covalently attached to the polypeptide. Also provided is a method for delivery of isradipine to a patient comprising administering to the patient a composition comprising a polypeptide and isradipine covalently attached to the polypeptide. Also provided is a method for protecting isradipine from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of isradipine from a composition comprising covalently attaching it to the polypeptide.

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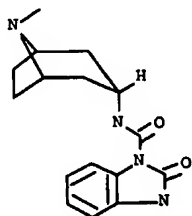
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING ITASETRON AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to itasetron, as well as methods for protecting and administering itasetron. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

15 Itasetron is a known pharmaceutical agent that is used in the treatment of emesis and anxiety. Its chemical name is endo-2,3-dihydro-N-(8-methyl-8-azabicyclo[3.2.1]oct-3-yl)-2-oxo-1H-benzimidazole-1-carboxamide. Its structure is:



20 The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase
5 markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly
10 acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the
15 active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and
20 aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can
25 also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage

reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in

the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (itasetron) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching itasetron to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising itasetron microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and itasetron covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Itasetron preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a

carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting itasetron from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering itasetron to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, itasetron is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, itasetron is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and itasetron is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, itasetron is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, itasetron is released from the composition in a sustained release.

In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

5 The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

(a) attaching itasetron to a side chain of an amino acid to form an active agent/amino acid complex;

10 (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and

(c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a
15 second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, itasetron and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular
20 transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the
25 glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is

described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

5 The present invention provides several benefits for active agent delivery. First, the invention can stabilize itasetron and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of itasetron. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

10 Itasetron is the subject of EP 309423 B (1994), herein incorporated by reference, which describes how to make that drug.

The composition of the invention comprises itasetron covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more
15 naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the
20 local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

25 Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino

acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

5 The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with
10 protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal
15 packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

 Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released.
20 The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is
25 often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

 Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's

decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of

active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

15

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent

delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

5 The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an
10 amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example
15 above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

20 The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal
25 enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-
30 carboxyanhydride. This intermediate can then be polymerized, as described above, using

any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart
5 specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these
10 polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the
15 alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-
20 dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, itasetron is covalently attached to the polypeptide via the amine group.

The polypeptide carrier can be prepared using conventional techniques. A
25 preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-itasetron conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 itasetron covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein itasetron is covalently attached to a side
chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein itasetron is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing itasetron from said composition in a pH-dependent manner.

15 19. A method for protecting itasetron from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of itasetron from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching itasetron to said polypeptide.

20 21. A method for delivering itasetron to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
itasetron covalently attached to said polypeptide.

25 22. The method of claim 21 wherein itasetron is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein itasetron is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

- 5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and itasetron covalently attached to the polypeptide. Also provided is a method for delivery of itasetron to a patient comprising administering to the patient a composition comprising a polypeptide and itasetron covalently attached to the polypeptide. Also provided is a method for protecting itasetron from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of itasetron from a composition comprising covalently attaching it to the polypeptide.

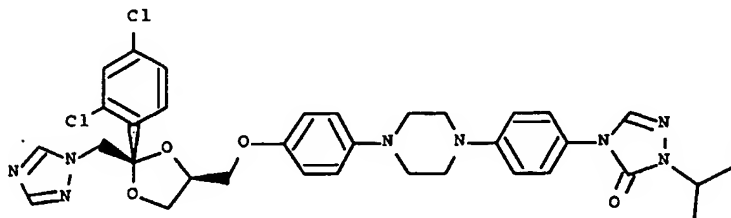
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING ITRACONAZOLE AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to itraconazole, as well as methods for protecting and administering itraconazole. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

 Itraconazole is a known pharmaceutical agent that is used in the treatment of
15 mycosis. Its chemical name is 4-[4-[4-[4-[[2-(2,4-dichlorophenyl)-2-[(1H-1,2,4-triazol-1-yl)methyl]-1,3-dioxolan-4-yl]methoxy]phenyl]-1-piperazinyl]phenyl]-2,4-dihydro-2-(1-methylpropyl)-3H-1,2,4-triazol-3-one. Its structure is:



 The novel pharmaceutical compound of the present invention is useful in
20 accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical
25 agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase
5 markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly
10 acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the
15 active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and
20 aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can
25 also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage

reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in

the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (itraconazole) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching itraconazole to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising itraconazole microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and itraconazole covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Itaconazole preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a

carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting itraconazole from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering itraconazole to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, itraconazole is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, itraconazole is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and itraconazole is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, itraconazole is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, itraconazole is released from the

composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic
5 release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching itraconazole to a side chain of an amino acid to form an active
10 agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

15 In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, itraconazole and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and
20 wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a
25 carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention.
30 The general applications of this invention to other active pharmaceutical agents is

described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

5 The present invention provides several benefits for active agent delivery. First, the invention can stabilize itraconazole and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of itraconazole. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

10 Itraconazole is the subject of U.S. Patent Numbers 4,267,179, 4,727,064, and 5,707,975, herein incorporated by reference, which describes how to make that drug.

15 The composition of the invention comprises itraconazole covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

20 Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

25 Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino

acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

5 The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with
10 protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal
15 packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

 Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released.
20 The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is
25 often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

 Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's

decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of

active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

15

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent

delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

5 The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an
10 amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example
15 above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

20 The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal
25 enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using
30

any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart
5 specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these
10 polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the
15 alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-
20 dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, the active agent is covalently attached to the polypeptide via a linker. This linker may be a small molecule containing 2-6 carbons and one or more functional groups (such as amines, amides, alcohols, or acids) or may be made up of a
25 short chain of either amino acids or carbohydrates.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention; a pre-first order release of the active agent is imparted by microencapsulating
5 the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect
10 that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own
15 associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more
20 adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance
25 absorption of the peptides.

Preferably, the resultant peptide-itraconazole conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5 several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10 followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15 the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20 a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
25 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 itraconazole covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein itraconazole is covalently attached to a
side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein itraconazole is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing itraconazole from said composition in a pH-dependent manner.

15 19. A method for protecting itraconazole from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of itraconazole from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching itraconazole to said polypeptide.

20 21. A method for delivering itraconazole to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
itraconazole covalently attached to said polypeptide.

25 22. The method of claim 21 wherein itraconazole is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein itraconazole is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and itraconazole covalently attached to the polypeptide. Also provided is a method for delivery of itraconazole to a patient comprising administering to the patient a composition comprising a polypeptide and
5 itraconazole covalently attached to the polypeptide. Also provided is a method for protecting itraconazole from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of itraconazole from a composition comprising covalently attaching it to the polypeptide.

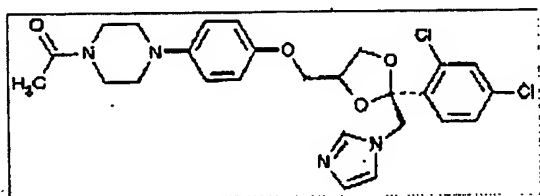
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING KETOCONAZOLE AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to ketoconazole, as well as methods for protecting and administering ketoconazole. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

 Ketoconazole is used in the treatment of blastomycosis, candidal infections (i.e.,
15 oropharyngeal and/or esophageal candidiasis, vulvovaginal candidiasis, candiduria, chronic mucocutaneous candidiasis), chromomycosis (chromoblastomycosis), coccidioidomycosis, histoplasmosis, and paracoccidioidomycosis. Its structure is:



 The novel pharmaceutical compound of the present invention is useful in
20 accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical
25 agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase
5 markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly
10 acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the
15 active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and
20 aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can
25 also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage

reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in

the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (ketoconazole) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching ketoconazole to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising ketoconazole microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and ketoconazole covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Ketoconazole preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a

carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting ketoconazole from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering ketoconazole to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, ketoconazole is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, ketoconazole is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and ketoconazole is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, ketoconazole is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, ketoconazole is released from the

composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic
5 release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching ketoconazole to a side chain of an amino acid to form an active
10 agent/amino acid complex;
 - (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
 - (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).
- 15 In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, ketoconazole and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and
20 wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a
25 carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention.
30 The general applications of this invention to other active pharmaceutical agents is

described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

5 The present invention provides several benefits for active agent delivery. First, the invention can stabilize ketoconazole and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of ketoconazole. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

10 The composition of the invention comprises ketoconazole covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or
15 more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the
20 conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding
25 are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded

protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational
5 entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant
10 force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has
15 maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is
20 a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious
25 chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

5 Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

10 Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

15 Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

20 As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of
25 active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant

groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence,

maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action
5 for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is
10 distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or
15 the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazenes to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

20 In the present invention, the active agent is covalently attached to the polypeptide via a linker. This linker may be a small molecule containing 2-6 carbons and one or more functional groups (such as amines, amides, alcohols, or acids) or may be made up of a short chain of either amino acids or carbohydrates.

The polypeptide carrier can be prepared using conventional techniques. A
25 preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-ketoconazole conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5 several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10 followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15 the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20 a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
25 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 ketoconazole covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein ketoconazole is covalently attached to a
side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein ketoconazole is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing ketoconazole from said composition in a pH-dependent manner.

15 19. A method for protecting ketoconazole from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of ketoconazole from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching ketoconazole to said polypeptide.

20 21. A method for delivering ketoconazole to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
ketoconazole covalently attached to said polypeptide.

25 22. The method of claim 21 wherein ketoconazole is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein ketoconazole is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

- 5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and ketoconazole covalently attached to the polypeptide. Also provided is a method for delivery of ketoconazole to a patient comprising administering to the patient a composition comprising a polypeptide and ketoconazole covalently attached to the polypeptide. Also provided is a method for protecting ketoconazole from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of ketoconazole from a composition comprising covalently attaching it to the polypeptide.

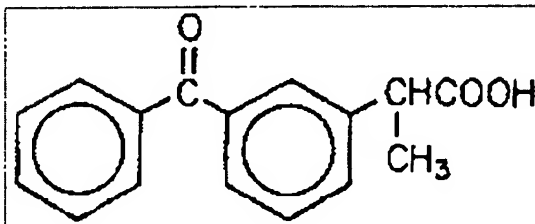
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING KETOPROFEN AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to ketoprofen, as well as methods for protecting and administering ketoprofen. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

 Ketoprofen is a known pharmaceutical agent that is used in the treatment of
15 arthritis and pain. Its structure is:



 The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered
20 product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

 Active agent delivery systems are often critical for the effective delivery of a
25 biologically active agent (active agent) to the appropriate target. The importance of these

systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf
5 life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as
10 resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the
15 stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human
20 growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties
25 to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble

microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients.

Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

5 The present invention provides covalent attachment of the active agent (ketoprofen) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching ketoprofen to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the
10 polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This
15 enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising ketoprofen microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and ketoprofen covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide,
20 (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

25 Ketoprofen preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to

the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

5 The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

10 Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

15 The invention also provides a method for protecting ketoprofen from degradation comprising covalently attaching it to a polypeptide.

 The invention also provides a method for delivering ketoprofen to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the
20 polypeptide. In a preferred embodiment, ketoprofen is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, ketoprofen is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and ketoprofen is released from the composition by dissolution
25 of the microencapsulating agent. In another preferred embodiment, ketoprofen is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, ketoprofen is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the

composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching ketoprofen to a side chain of an amino acid to form an active agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, ketoprofen and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize ketoprofen and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of ketoprofen.

- 5 Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

- The composition of the invention comprises ketoprofen covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one
10 of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

- Proteins, oligopeptides and polypeptides are polymers of amino acids that have
15 primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains
20 constitute the tertiary structure.

- Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino
25 acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be

enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding.

Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will
5 ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of
10 the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a
15 synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level
20 of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond
25 between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate

weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border
 5 membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

10

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For
 15 example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could
 20 conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, ketoprofen is covalently attached to the polypeptide via the carboxylic acid group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized
5 adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions,
10 sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is
15 particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

20 Preferably, the resultant peptide-ketoprofen conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

25 An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product

precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

- The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C.
- 5 The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

- 10 In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product
- 15 is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

- Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include
- 20 dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

- There have been over 30 different γ -alkyl glutamates prepared any one of which
- 25 may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for

several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C.

- 5 The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

- 10 γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

- 15 γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

- 20 Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 ketoprofen covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein ketoprofen is covalently attached to a side
chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein ketoprofen is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing ketoprofen from said composition in a pH-dependent manner.

15 19. A method for protecting ketoprofen from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of ketoprofen from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching ketoprofen to said polypeptide.

20 21. A method for delivering ketoprofen to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
ketoprofen covalently attached to said polypeptide.

25 22. The method of claim 21 wherein ketoprofen is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein ketoprofen is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and ketoprofen covalently attached to the polypeptide. Also provided is a method for delivery of ketoprofen to a patient comprising administering to the patient a composition comprising a polypeptide and
5 ketoprofen covalently attached to the polypeptide. Also provided is a method for protecting ketoprofen from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of ketoprofen from a composition comprising covalently attaching it to the polypeptide.

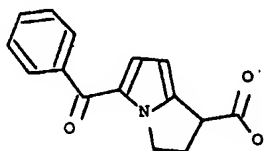
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING KETOROLAC AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to ketorolac, as well as methods for protecting and administering ketorolac. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

 Ketorolac is a known pharmaceutical agent that is used in the treatment of pain.
15 Its chemical name is (+,-)-5-benzoyl-2,3-dihydro-1H-pyrrolizine-1-carboxylic acid. Its structure is:



 The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability
20 of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

25 Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these

systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf
5 life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as
10 resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the
15 stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human
20 growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties
25 to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble

microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients.

Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

5 The present invention provides covalent attachment of the active agent (ketorolac) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching ketorolac to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will
10 stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a
15 second order sustained release mechanism.

 Alternatively, the present invention provides a pharmaceutical composition comprising ketorolac microencapsulated by a polypeptide.

 The invention provides a composition comprising a polypeptide and ketorolac covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide,
20 (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

25 Ketorolac preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to

the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

5 The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

10 Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

15 The invention also provides a method for protecting ketorolac from degradation comprising covalently attaching it to a polypeptide.

 The invention also provides a method for delivering ketorolac to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, ketorolac is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, ketorolac is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and ketorolac is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, ketorolac is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, ketorolac is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is

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controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method
5 comprises the steps of:

(a) attaching ketorolac to a side chain of an amino acid to form an active agent/amino acid complex;

(b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and

10 (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, ketorolac and a second active agent can be copolymerized in step (c). In another
15 preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine,
20 cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

25 It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize ketorolac and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of ketorolac. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

Ketorolac is the subject of GB 1554057 (1979), based on priority application US 704909 (1976), herein incorporated by reference, which describes how to make that drug.

The composition of the invention comprises ketorolac covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded

protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational
5 entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular
10 bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has
15 maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is
20 a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious
25 chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

5 Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

10 Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

15 Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

20 As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of
25 active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant

groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence,

maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action
5 for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is
10 distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or
15 the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

20 In the present invention, ketorolac is covalently attached to the polypeptide via the carboxylic acid group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer
25 can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the

invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

5 There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the
10 transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active
15 agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the
20 catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-ketorolac conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

25 Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5 several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10 followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15 the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20 a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
25 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 ketorolac covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein ketorolac is covalently attached to a side
chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein ketorolac is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing ketorolac from said composition in a pH-dependent manner.

15 19. A method for protecting ketorolac from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of ketorolac from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching ketorolac to said polypeptide.

20 21. A method for delivering ketorolac to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
ketorolac covalently attached to said polypeptide.

25 22. The method of claim 21 wherein ketorolac is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein ketorolac is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and ketorolac covalently attached to the polypeptide. Also provided is a method for delivery of ketorolac to a patient comprising administering to the patient a composition comprising a polypeptide and ketorolac covalently attached to the polypeptide. Also provided is a method for protecting ketorolac from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of ketorolac from a composition comprising covalently attaching it to the polypeptide.

A NOVEL PHARMACEUTICAL COMPOUND CONTAINING KETOTIFEN AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to ketotifen, as well as methods for protecting and administering ketotifen. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

 Ketotifen is a known pharmaceutical agent that is used in the treatment of allergic
15 conjunctivitis.

 The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type;
20 and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

 Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these
25 systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf

life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is

unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically
5 require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous
10 pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The
15 released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that
20 incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly
25 or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited
30 to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (ketotifen) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching ketotifen to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising ketotifen microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and ketotifen covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Ketotifen preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting ketotifen from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering ketotifen to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, ketotifen is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, ketotifen is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and ketotifen is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, ketotifen is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, ketotifen is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching ketotifen to a side chain of an amino acid to form an active agent/amino acid complex;
 - (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
 - (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).
- In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, ketotifen and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

- It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention.
- The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

- The present invention provides several benefits for active agent delivery. First, the invention can stabilize ketotifen and prevent its digestion in the stomach. In addition,

the pharmacologic effect can be prolonged by delayed release of ketotifen. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

5 The composition of the invention comprises ketotifen covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or
10 more naturally occurring amino acids and one or more synthetic amino acids.

 Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the
15 conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

 Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding
20 are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best
25 model for determining forces contributing to protein stability is the solid reference state.

 The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior

and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are
5 "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

10 Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only
15 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

20 Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For
25 instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding.
Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will

ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all
5 be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple
10 active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain
15 length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of
20 active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate
25 weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the

jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

5

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order

to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example
5 above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

10 The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal
15 enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using
20 any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart
25 specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these
30 polypeptides through a spacer or linker on the pendant group, which is terminated,

preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, ketotifen is covalently attached to the polypeptide via a hydroxyl, amine or carboxylic acid group or, alternatively, via an artificial linker..

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known

intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions,
5 sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is
10 particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

15 Preferably, the resultant peptide-ketotifen conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

20 An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or
25 dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product
5 precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product,
10 which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and
15 filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 ketotifen covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
15 one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein ketotifen is covalently attached to a side
chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
10. The composition of claim 9 wherein said microencapsulating agent is
20 selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein ketotifen is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing ketotifen from said composition in a pH-dependent manner.

15 19. A method for protecting ketotifen from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of ketotifen from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching ketotifen to said polypeptide.

20 21. A method for delivering ketotifen to a patient comprising administering to said patient a composition comprising:
 a polypeptide; and
 ketotifen covalently attached to said polypeptide.

25 22. The method of claim 21 wherein ketotifen is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein ketotifen is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and ketotifen covalently attached to the polypeptide. Also provided is a method for delivery of ketotifen to a patient comprising administering to the patient a composition comprising a polypeptide and ketotifen covalently attached to the polypeptide. Also provided is a method for protecting ketotifen from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of ketotifen from a composition comprising covalently attaching it to the polypeptide.

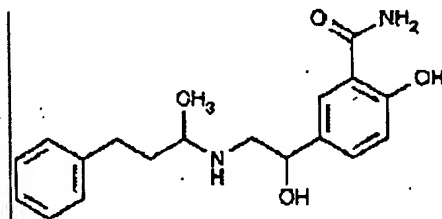
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING LABETALOL AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to labetalol, as well as methods for protecting and administering labetalol. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

 Labetalol is a known pharmaceutical agent that is used in the treatment of
15 hypertension. Its structure is:



 The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered
20 product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

 Active agent delivery systems are often critical for the effective delivery of a
25 biologically active agent (active agent) to the appropriate target. The importance of these

systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf
5 life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as
10 resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the
15 stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human
20 growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties
25 to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble

microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients.

Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

5 The present invention provides covalent attachment of the active agent (labetalol) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching labetalol to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will
10 stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a
15 second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising labetalol microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and labetalol covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide,
20 (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

25 Labetalol preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to

the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

5 The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

10 Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

15 The invention also provides a method for protecting labetalol from degradation comprising covalently attaching it to a polypeptide.

 The invention also provides a method for delivering labetalol to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, labetalol is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, labetalol is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and labetalol is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, labetalol is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, labetalol is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is

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controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method
5 comprises the steps of:

(a) attaching labetalol to a side chain of an amino acid to form an active agent/amino acid complex;

(b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and

10 (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, labetalol and a second active agent can be copolymerized in step (c). In another
15 preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine,
20 cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

25 It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize labetalol and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of labetalol. Furthermore, 5 active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

The composition of the invention comprises labetalol covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one 10 of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have 15 primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains 20 constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino 25 acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be

enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will
5 ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of
10 the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a
15 synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level
20 of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond
25 between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate

weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border
 5 membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

10

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For
 15 example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could
 20 conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, labetalol is covalently attached to the polypeptide via the hydroxyl group:

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized
5 adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions,
10 sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is
15 particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

20 Preferably, the resultant peptide-labetalol conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

25 An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product

precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C.

- 5 The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

- 10 In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product
15 is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

- Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include
20 dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

- There have been over 30 different γ -alkyl glutamates prepared any one of which
25 may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for

several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

- The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C.
- 5 The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

- 10 γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

- 15 γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

- 20 Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 labetalol covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
15 one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein labetalol is covalently attached to a side
chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 10 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein labetalol is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing labetalol from said composition in a pH-dependent manner.

15 19. A method for protecting labetalol from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of labetalol from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching labetalol to said polypeptide.

20 21. A method for delivering labetalol to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
labetalol covalently attached to said polypeptide.

25 22. The method of claim 21 wherein labetalol is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein labetalol is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and labetalol covalently attached to the polypeptide. Also provided is a method for delivery of labetalol to a patient comprising administering to the patient a composition comprising a polypeptide and labetalol covalently attached to the polypeptide. Also provided is a method for protecting labetalol from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of labetalol from a composition comprising covalently attaching it to the polypeptide.

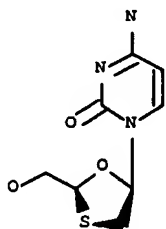
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING LAMIVUDINE AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to lamivudine, as well as methods for protecting and administering lamivudine. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

 Lamivudine is a known pharmaceutical agent that is used in the treatment of
15 hepatitis, viral infection and HIV infection. Its chemical name is (2R-cis)-4-amino-1-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-2(1H)-pyrimidinone. Its structure is:



 The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability
20 of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase
5 markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly
10 acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the
15 active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and
20 aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can
25 also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage

reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in

the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients.

Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (lamivudine) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching lamivudine to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising lamivudine microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and lamivudine covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Lamivudine preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a

carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting lamivudine from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering lamivudine to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, lamivudine is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, lamivudine is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and lamivudine is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, lamivudine is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, lamivudine is released from the composition in a

sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching lamivudine to a side chain of an amino acid to form an active agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, lamivudine and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is

described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

5 The present invention provides several benefits for active agent delivery. First, the invention can stabilize lamivudine and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of lamivudine. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

10 Lamivudine is the subject of U.S. Patent Numbers 5,047,407 and 5,905,082, herein incorporated by reference, which describes how to make that drug.

The composition of the invention comprises lamivudine covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more
15 naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the
20 local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

25 Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino

acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

5 The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with
10 protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal
15 packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

 Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released.
20 The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is
25 often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

 Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's

decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of

active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

15

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent

delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

5 The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an
10 amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example
15 above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

20 The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal
25 enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-
30 carboxyanhydride. This intermediate can then be polymerized, as described above, using

any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart
5 specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these
10 polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the
15 alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-
20 dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, lamivudine is covalently attached to the polypeptide via the hydroxyl group.

The polypeptide carrier can be prepared using conventional techniques. A
25 preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-lamivudine conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5 several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10 followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15 the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20 a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
25 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 lamivudine covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein lamivudine is covalently attached to a
side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein lamivudine is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing lamivudine from said composition in a pH-dependent manner.

15 19. A method for protecting lamivudine from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of lamivudine from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching lamivudine to said polypeptide.

20 21. A method for delivering lamivudine to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
lamivudine covalently attached to said polypeptide.

25 22. The method of claim 21 wherein lamivudine is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein lamivudine is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

- 5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and lamivudine covalently attached to the polypeptide. Also provided is a method for delivery of lamivudine to a patient comprising administering to the patient a composition comprising a polypeptide and
5 lamivudine covalently attached to the polypeptide. Also provided is a method for protecting lamivudine from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of lamivudine from a composition comprising covalently attaching it to the polypeptide.

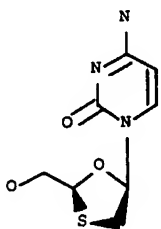
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING LAMIVUDINE AND ZIDOVUDINE AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

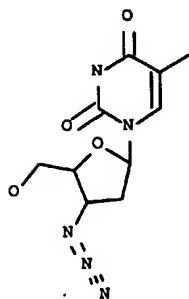
5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to lamivudine and zidovudine, as well as methods for protecting and administering lamivudine and zidovudine. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier
10 compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

 Lamivudine is a known pharmaceutical agent that is used in the treatment of hepatitis, viral infection and HIV infection. Its chemical name is (2R-cis)-4-amino-1-[2-
15 (hydroxymethyl)-1,3-oxathiolan-5-yl]-2(1H)-pyrimidinone. Its structure is:



 Zidovudine has the chemical name 3'-azido-3'-deoxythymidine. Its structure is



The two drugs together are used as a fixed-dose combination tablet comprising the reverse transcriptase inhibitors lamivudine and zidovudine for the treatment of HIV infection.

The novel pharmaceutical compound of the present invention is useful in
5 accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical
10 agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase
15 markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly
20 acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the
25 active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and

aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders

and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that
5 incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly
10 or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited
15 to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (lamivudine and zidovudine) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching
20 lamivudine and zidovudine to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide.
25 Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising lamivudine and zidovudine microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and lamivudine and zidovudine covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Lamivudine and zidovudine preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting lamivudine and zidovudine from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering lamivudine and zidovudine to a patient, the patient being a human or a non-human animal, comprising administering to
5 the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, lamivudine and zidovudine are released from the composition by an enzyme-catalyzed release. In another preferred embodiment, lamivudine and zidovudine are released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred
10 embodiment, the composition further comprises a microencapsulating agent and lamivudine and zidovudine are released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, lamivudine and zidovudine are released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, lamivudine and zidovudine are released from the
15 composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

20 The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching lamivudine and zidovudine to a side chain of an amino acid to form an active agent/amino acid complex;
- 25 (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a
30 second active agent. When steps (a) and (b) are repeated prior to step (c) with a second

agent, lamivudine and zidovudine and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize lamivudine and zidovudine and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of lamivudine and zidovudine. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

Lamivudine is the subject of U.S. Patent Numbers 5,047,407 and 5,905,082, herein incorporated by reference, which describes how to make that drug. Zidovudine is covered by EP 196185 B (1989). The combination is covered by the following U.S.

patents, which are hereby incorporated by reference: 4,724,232, 4,818,538, 4,828,838, 4,833,130, 4,837,208 and 6,113,920.

The composition of the invention comprises lamivudine and zidovudine covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with

protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and
5 contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a
10 peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and
15 at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of
20 the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be
25 enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will

ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all
5 be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple
10 active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain
15 length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of
20 active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate
25 weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the

jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

5

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order

to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example
5 above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

10 The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal
15 enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using
20 any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart
25 specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these
30 polypeptides through a spacer or linker on the pendant group, which is terminated,

preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, lamivudine and zidovudine are covalently attached to the polypeptide via the hydroxyl group on each.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known

intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions,
5 sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is
10 particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

15 Preferably, the resultant peptide-lamivudine and zidovudine conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

20 **Acid/N-terminus conjugation**

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product
25 precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product
5 precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product,
10 which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and
15 filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 lamivudine and zidovudine covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein lamivudine and zidovudine are covalently attached to a side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein lamivudine and zidovudine are conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing lamivudine and zidovudine from said composition in a pH-dependent manner.

15 19. A method for protecting lamivudine and zidovudine from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of lamivudine and zidovudine from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching lamivudine and zidovudine to said polypeptide.

20 21. A method for delivering lamivudine and zidovudine to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
lamivudine and zidovudine covalently attached to said polypeptide.

25 22. The method of claim 21 wherein lamivudine and zidovudine are released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein lamivudine and zidovudine are released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

10 Abstract

 A composition comprising a polypeptide and lamivudine and zidovudine covalently attached to the polypeptide. Also provided is a method for delivery of lamivudine and zidovudine to a patient comprising administering to the patient a composition comprising a polypeptide and lamivudine and zidovudine covalently
15 attached to the polypeptide. Also provided is a method for protecting lamivudine and zidovudine from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of lamivudine and zidovudine from a composition comprising covalently attaching it to the polypeptide.

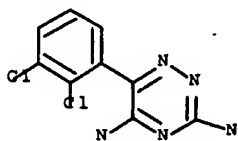
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING LAMOTRIGINE AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to lamotrigine, as well as methods for protecting and administering lamotrigine. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

 Lamotrigine is a known pharmaceutical agent that is used in the treatment of
15 epilepsy, psychosis and depression. Its chemical name is 6-(2,3-dichlorophenyl)-1,2,4-triazine-3,5-diamine. Its structure is:



 The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability
20 of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

25 Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these

systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf
5 life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as
10 resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the
15 stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human
20 growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties
25 to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble

microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients.

Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

5 The present invention provides covalent attachment of the active agent (lamotrigine) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching lamotrigine to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the
10 polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This
15 enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising lamotrigine microencapsulated by a polypeptide.

20 The invention provides a composition comprising a polypeptide and lamotrigine covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a
heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

25 Lamotrigine preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to

the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

5 The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

10 Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

15 The invention also provides a method for protecting lamotrigine from degradation comprising covalently attaching it to a polypeptide.

 The invention also provides a method for delivering lamotrigine to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the
20 polypeptide. In a preferred embodiment, lamotrigine is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, lamotrigine is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a
25 microencapsulating agent and lamotrigine is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, lamotrigine is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, lamotrigine is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant

from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching lamotrigine to a side chain of an amino acid to form an active agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, lamotrigine and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize lamotrigine and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of lamotrigine.

- 5 Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

Lamotrigine is the subject of U.S. Patent Numbers 4,602,017 and 5,698,226, herein incorporated by reference, which describes how to make that drug.

- 10 The composition of the invention comprises lamotrigine covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or
15 more naturally occurring amino acids and one or more synthetic amino acids.

- Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the
20 conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

- Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding
25 are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded

protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational
5 entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular
10 bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has
15 maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is
20 a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious
25 chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

5 Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

10 Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

15 Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

20 As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of
25 active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant

groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence,

maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action
5 for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is
10 distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or
15 the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

20 In the present invention, lamotrigine is covalently attached to the polypeptide via the amino group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer
25 can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the

invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-lamotrigine conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5 several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10 followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15 the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20 a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
25 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 lamotrigine covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein lamotrigine is covalently attached to a
side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein lamotrigine is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing lamotrigine from said composition in a pH-dependent manner.

15 19. A method for protecting lamotrigine from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of lamotrigine from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching lamotrigine to said polypeptide.

20 21. A method for delivering lamotrigine to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
lamotrigine covalently attached to said polypeptide.

25 22. The method of claim 21 wherein lamotrigine is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein lamotrigine is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and lamotrigine covalently attached to the polypeptide. Also provided is a method for delivery of lamotrigine to a patient comprising administering to the patient a composition comprising a polypeptide and
5 lamotrigine covalently attached to the polypeptide. Also provided is a method for protecting lamotrigine from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of lamotrigine from a composition comprising covalently attaching it to the polypeptide.

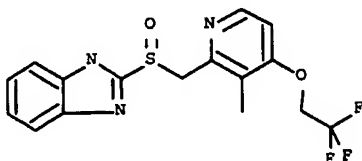
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING LANSOPRAZOLE AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to lansoprazole, as well as methods for protecting and administering lansoprazole. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

Lansoprazole is a known pharmaceutical agent that is used in the treatment of
15 ulcer and bacterial infection. Its chemical name is 2-[[[3-methyl-4-(2,2,2-trifluoroethoxy)-2-pyridinyl]methyl]sulfinyl]-1H-benzimidazole. Its structure is:



The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability
20 of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase
5 markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly
10 acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the
15 active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and
20 aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can
25 also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage

reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in

the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (lansoprazole) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching lansoprazole to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising lansoprazole microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and lansoprazole covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Lansoprazole preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a

carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting lansoprazole from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering lansoprazole to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, lansoprazole is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, lansoprazole is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and lansoprazole is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, lansoprazole is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, lansoprazole is released from the

composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic
5 release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching lansoprazole to a side chain of an amino acid to form an active
10 agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

15 In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, lansoprazole and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and
20 wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a
25 carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention.
30 The general applications of this invention to other active pharmaceutical agents is

described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize lansoprazole and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of lansoprazole. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

Lansoprazole is the subject of U.S. Patent Number 4628098, 4689333, 5026560, 5045321, 5093132 and 5433959, herein incorporated by reference, which describes how to make that drug.

The composition of the invention comprises lansoprazole covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a

particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best
5 model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior
10 and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and
15 contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a
20 peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and
25 at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

5 Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding.
10 Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all
15 be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple
20 active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain
25 length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the

carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a
 5 macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several
 10 aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of
 15 lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyridoxine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's
 20 molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular

weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could
5 conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-
10 terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the
15 preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the
20 peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid
25 moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively,
30 the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-

carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, lansoprazole is covalently attached to the polypeptide via the amino group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-lansoprazole conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5 several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10 followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15 the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20 a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
25 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 lansoprazole covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein lansoprazole is covalently attached to a
side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein lansoprazole is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing lansoprazole from said composition in a pH-dependent manner.

15 19. A method for protecting lansoprazole from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of lansoprazole from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching lansoprazole to said polypeptide.

20 21. A method for delivering lansoprazole to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
lansoprazole covalently attached to said polypeptide.

25 22. The method of claim 21 wherein lansoprazole is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein lansoprazole is released from said composition by a pH-dependent unfolding of said polypeptide.
24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.
- 5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and lansoprazole covalently attached to the polypeptide. Also provided is a method for delivery of lansoprazole to a patient comprising administering to the patient a composition comprising a polypeptide and
5 lansoprazole covalently attached to the polypeptide. Also provided is a method for protecting lansoprazole from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of lansoprazole from a composition comprising covalently attaching it to the polypeptide.

A NOVEL PHARMACEUTICAL COMPOUND CONTAINING LANSOPRAZOLE, AMOXICILLIN AND CLARITHROMICIN AND METHODS OF MAKING AND USING SAME

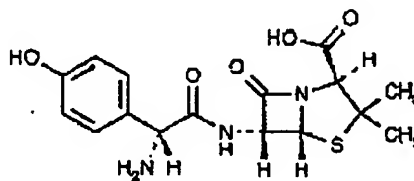
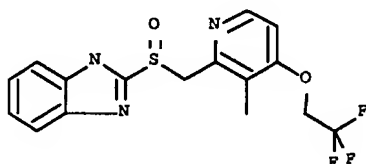
5 FIELD OF THE INVENTION

The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to lansoprazole, amoxicillin and clarithromycin, as well as methods for protecting and administering lansoprazole, amoxicillin and clarithromycin. This novel compound, referred to as a

10 CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

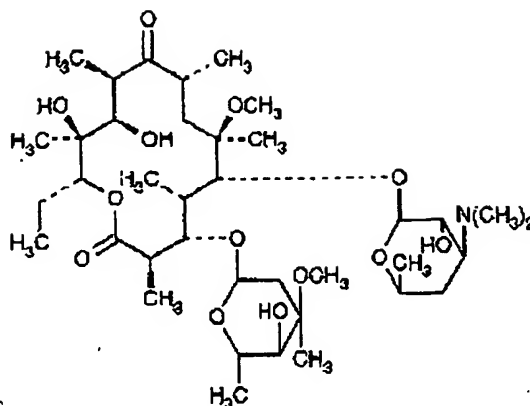
15 BACKGROUND OF THE INVENTION

Lansoprazole, amoxicillin and clarithromycin are used together in the treatment of duodenal ulcer. Lansoprazole's chemical name is 2-[[[3-methyl-4-(2,2,2-trifluoroethoxy)-2-pyridinyl]methyl]sulfinyl]-1H-benzimidazole. Its structure is:



20

The structure of amoxicillin is



The structure of clarithromycin is

The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of

cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the
5 stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human
10 growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties
15 to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble
20 microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

25 In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral

administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently
5 attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology
10 combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

15 It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high
20 moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

25 The present invention provides covalent attachment of the active agent (lansoprazole, amoxicillin and clarithromycin) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching lansoprazole, amoxicillin and clarithromycin to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide,

also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous
5 enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising lansoprazole, amoxicillin and clarithromycin microencapsulated by a
10 polypeptide.

The invention provides a composition comprising a polypeptide and lansoprazole, amoxicillin and clarithromycin covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino
15 acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Lansoprazole, amoxicillin and clarithromycin preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred
20 embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an
25 alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino

acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestable tablet, an intravenous preparation or an oral suspension. The active agent can be

5 conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting lansoprazole, amoxicillin and clarithromycin from degradation comprising covalently attaching it to a polypeptide.

10 The invention also provides a method for delivering lansoprazole, amoxicillin and clarithromycin to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, lansoprazole, amoxicillin and clarithromycin are released from the composition by an enzyme-catalyzed

15 release. In another preferred embodiment, lansoprazole, amoxicillin and clarithromycin are released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and lansoprazole, amoxicillin and clarithromycin are released from the composition by dissolution of the microencapsulating agent. In another

20 preferred embodiment, lansoprazole, amoxicillin and clarithromycin are released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, lansoprazole, amoxicillin and clarithromycin are released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and

25 release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- 5 (a) attaching lansoprazole, amoxicillin and clarithromycin to a side chain of an amino acid to form an active agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

10 In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, lansoprazole, amoxicillin and clarithromycin and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis
15 of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an
20 ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

25 It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize lansoprazole, amoxicillin and clarithromycin and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by
5 delayed release of lansoprazole, amoxicillin and clarithromycin. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

The composition of the invention comprises lansoprazole, amoxicillin and
10 clarithromycin covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or
15 (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the
20 conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding
25 are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded

protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational
5 entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular
10 bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has
15 maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is
20 a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious
25 chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

5 Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

10 Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

15 Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

20 As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of
25 active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant

groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence,

maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action
5 for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is
10 distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or
15 the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

20 In the present invention, lansoprazole, amoxicillin and clarithromycin are covalently attached to the polypeptide via the hydroxyl and or amino groups.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer
25 can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the

invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-lansoprazole, amoxicillin and clarithromycin conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5 several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10 followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15 the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20 a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
25 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 lansoprazole, amoxicillin and clarithromycin covalently attached to said
polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
- 10 4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
15 two or more synthetic amino acids.
7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein lansoprazole, amoxicillin and
clarithromycin are covalently attached to a side chain, the N-terminus or the C-terminus
20 of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.

11. The composition of claim 1 further comprising an adjuvant.
12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.
13. The composition of claim 1 further comprising a pharmaceutically acceptable
5 excipient.
14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.
15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.
- 10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.
17. The composition of claim 1 wherein lansoprazole, amoxicillin and clarithromycin are conformationally protected by folding of said polypeptide about said active agent.
- 15 18. The composition of claim 1 wherein said polypeptide is capable of releasing lansoprazole, amoxicillin and clarithromycin from said composition in a pH-dependent manner.
19. A method for protecting lansoprazole, amoxicillin and clarithromycin from degradation comprising covalently attaching said active agent to a polypeptide.
- 20 20. A method for controlling release of lansoprazole, amoxicillin and clarithromycin from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching lansoprazole, amoxicillin and clarithromycin to said polypeptide.
21. A method for delivering lansoprazole, amoxicillin and clarithromycin to a
25 patient comprising administering to said patient a composition comprising:

a polypeptide; and

lansoprazole, amoxicillin and clarithromycin covalently attached to said polypeptide.

22. The method of claim 21 wherein lansoprazole, amoxicillin and clarithromycin
5 are released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein lansoprazole, amoxicillin and clarithromycin are released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

10 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

15 A composition comprising a polypeptide and lansoprazole, amoxicillin and clarithromycin covalently attached to the polypeptide. Also provided is a method for delivery of lansoprazole, amoxicillin and clarithromycin to a patient comprising administering to the patient a composition comprising a polypeptide and lansoprazole, amoxicillin and clarithromycin covalently attached to the polypeptide. Also provided is a
20 method for protecting lansoprazole, amoxicillin and clarithromycin from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of lansoprazole, amoxicillin and clarithromycin from a composition comprising covalently attaching it to the polypeptide.

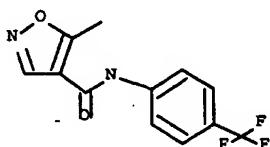
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING LEFLUNOMIDE AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to leflunomide, as well as methods for protecting and administering leflunomide. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

 Leflunomide is a known pharmaceutical agent that is used in the treatment of
15 rheumatoid arthritis. Its chemical name is 5-methyl-N-[4-(trifluoromethyl)phenyl]-4-isoxazolecarboxamide. Its structure is:



 The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability
20 of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

25 Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these

systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf
5 life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as
10 resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the
15 stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human
20 growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties
25 to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble

microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients.

Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

5 The present invention provides covalent attachment of the active agent (leflunomide) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching leflunomide to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the
10 polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This
15 enzymatic action introduces a second order sustained release mechanism.

 Alternatively, the present invention provides a pharmaceutical composition comprising leflunomide microencapsulated by a polypeptide.

 The invention provides a composition comprising a polypeptide and leflunomide covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide,
20 (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

25 Leflunomide preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to

the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

5 The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

10 Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

15 The invention also provides a method for protecting leflunomide from degradation comprising covalently attaching it to a polypeptide.

 The invention also provides a method for delivering leflunomide to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the
20 polypeptide. In a preferred embodiment, leflunomide is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, leflunomide is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and leflunomide is released from the composition by
25 dissolution of the microencapsulating agent. In another preferred embodiment, leflunomide is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, leflunomide is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and

release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching leflunomide to a side chain of an amino acid to form an active agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, leflunomide and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize leflunomide and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of leflunomide.

5 Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

Leflunomide is the subject of U.S. Patent Number 5679709, herein incorporated by reference, which describes how to make that drug.

10 The composition of the invention comprises leflunomide covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or

15 more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the

20 conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding

25 are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded

protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational
5 entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular
10 bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has
15 maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is
20 a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious
25 chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

- 5 Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

- 10 Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

- 15 Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

- 20 As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.
- 25

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant

groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence,

maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action
5 for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is
10 distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or
15 the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

20 In the present invention, the active agent is covalently attached to the polypeptide via a linker. This linker may be a small molecule containing 2-6 carbons and one or more functional groups (such as amines, amides, alcohols, or acids) or may be made up of a short chain of either amino acids or carbohydrates.

The polypeptide carrier can be prepared using conventional techniques. A
25 preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-leflunomide conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5 several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10 followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15 the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20 a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
25 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 leflunomide covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein leflunomide is covalently attached to a
side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein leflunomide is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing leflunomide from said composition in a pH-dependent manner.

15 19. A method for protecting leflunomide from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of leflunomide from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching leflunomide to said polypeptide.

20 21. A method for delivering leflunomide to a patient comprising administering to said patient a composition comprising:

 a polypeptide; and
 leflunomide covalently attached to said polypeptide.

25 22. The method of claim 21 wherein leflunomide is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein leflunomide is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

- 5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and leflunomide covalently attached to the polypeptide. Also provided is a method for delivery of leflunomide to a patient comprising administering to the patient a composition comprising a polypeptide and
5 leflunomide covalently attached to the polypeptide. Also provided is a method for protecting leflunomide from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of leflunomide from a composition comprising covalently attaching it to the polypeptide.

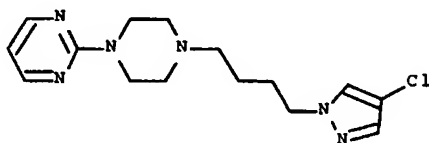
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING LESOPITRON AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to lesopitron, as well as methods for protecting and administering lesopitron. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

Lesopitron is a known pharmaceutical agent that is used in the treatment of
15 anxiety. Its chemical name is 2-[4-[4-(4-chloro-1H-pyrazol-1-yl)butyl]-1-piperazinyl]pyrimidine dihydrochloride. Its structure is:



The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability
20 of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

25 Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these

systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf
5 life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as
10 resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the
15 stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human
20 growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties
25 to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble

microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients.

Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

5 The present invention provides covalent attachment of the active agent (lesopitron) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching lesopitron to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the
10 polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This
15 enzymatic action introduces a second order sustained release mechanism.

 Alternatively, the present invention provides a pharmaceutical composition comprising lesopitron microencapsulated by a polypeptide.

 The invention provides a composition comprising a polypeptide and lesopitron covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide,
20 (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

25 Lesopitron preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to

the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

5 The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

10 Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

15 The invention also provides a method for protecting lesopitron from degradation comprising covalently attaching it to a polypeptide.

 The invention also provides a method for delivering lesopitron to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the
20 polypeptide. In a preferred embodiment, lesopitron is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, lesopitron is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and lesopitron is released from the composition by dissolution
25 of the microencapsulating agent. In another preferred embodiment, lesopitron is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, lesopitron is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is

controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method
5 comprises the steps of:

(a) attaching lesopitron to a side chain of an amino acid to form an active agent/amino acid complex;

(b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and

10 (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, lesopitron and a second active agent can be copolymerized in step (c). In another
15 preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine,
20 cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

25 It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize lesopitron and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of lesopitron.

5 Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

Lesopitron is the subject of EP 382637 A (1990), herein incorporated by reference, which describes how to make that drug.

10 The composition of the invention comprises lesopitron covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or

15 more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the

20 conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding

25 are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded

protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational
5 entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular
10 bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has
15 maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is
20 a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious
25 chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

5 Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

10 Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

15 Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

20 As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of
25 active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant

groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence,

maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action
5 for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is
10 distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or
15 the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

20 In the present invention, the active agent is covalently attached to the polypeptide via a linker. This linker may be a small molecule containing 2-6 carbons and one or more functional groups (such as amines, amides, alcohols, or acids) or may be made up of a short chain of either amino acids or carbohydrates.

The polypeptide carrier can be prepared using conventional techniques. A
25 preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-lesopitron conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5 several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10 followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15 the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20 a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
25 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
lesopitron covalently attached to said polypeptide.
5
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein lesopitron is covalently attached to a side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein lesopitron is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing lesopitron from said composition in a pH-dependent manner.

15 19. A method for protecting lesopitron from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of lesopitron from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching lesopitron to said polypeptide.

20 21. A method for delivering lesopitron to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
lesopitron covalently attached to said polypeptide.

25 22. The method of claim 21 wherein lesopitron is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein lesopitron is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.